

Cultural divides, forty years on

A famous lecture given in 1959 still resonates. Although time has eroded many of the cultural fissures that it addressed, current debates about biotechnology highlight continuing problems of mutual incomprehension.

No one can now complain, as C. P. Snow did 40 years ago in his lecture *The Two Cultures*, of a lack of assimilation of science by the arts. There is now too much success in the form of plays, novels, poetry and paintings that have been infused with science, and have wide appeal, for that basic concern to be sustained.

But Snow's worries about a divided culture were of much broader scope. His starting point was that the "traditional" literary culture not only failed to understand science, but was also too dominant. "Scientists have the future in their bones, traditional culture responds by wishing that the future does not exist. It is the traditional culture ... which manages the western world." However accurate his portrayal then, society's hierarchy has moved on. Few Western administrations and governments suffer fundamentally from a lack of scientific and technological perspective, or from another sin that he highlighted, a lack of serious interest in engineering and business.

Snow placed great faith in an improved balance of education as the critical way forward. The fact that surveys of the public continue to display a worryingly low level of knowledge of scientific facts, or comprehension of the scientific process, suggests that undoubted progress in lessening cultural divides has been as much due to other factors.

Here, scientists willing to communicate widely, science writers and publishers should take their bow. Alongside the quality media, one can celebrate books like *Lucy* (written by Donald Johanson), which conveys not only the excitement of palaeontology but also its cautious thoroughness; like *Nobel Dreams* (Gary Taubes), which illuminates the influence of personality in the practice of particle physics; like *Guns, Germs and Steel* (Jared Diamond), which highlights what science can contribute to understanding 13,000 years of human history — these as well as the cornucopia of more explanatory texts on genetics, evolution, cosmology and quantum physics can take their share of the credit. And if novels such as *Mendel's Dwarf* (Simon Mawer) and plays such as *Copenhagen* (Michael Frayn) not only assimilate science but explicitly explore issues that it raises, so much the better for cultural bridge building.

Disparity

There is one point where Snow turned out to be mistaken: his prediction that, once it was clear how "rather easy" it is to take up technology, the poor nations of the world would refuse to sit still and, using whatever aid they could obtain, would catch up. "The disparity between rich and poor has been noticed ... Whatever else lasts until 2000, that won't." But how would the gap be removed, and by whom? Large amounts of capital, Snow said, must come from outside, by way of national efforts. An immense investment of capital and scientists into poor countries by the West, with negligible rewards in the short term and uncertain benefits in the long; Snow acknowledged that this was politically daunting but believed it essential. The political and economic reasons why Snow's prediction proved wrong sound a warning against optimistic technocratic naivety.

But there is a sharp end of public controversy about technologies where principles and vested interests are at stake, where science is uncertain, where public confidence is even more uncertain, and where Snow's concerns about scientific incomprehension and, for that mat-

ter, about non-developed nations, are as relevant as ever. Nowhere has this been more vividly displayed than in debates about the use of genetically modified organisms, especially in Europe and parts of the developing world. "...total incomprehension gives, much more pervasively than we realise, living in it, an unscientific flavour to the whole culture, and that unscientific flavour is often, much more often than we admit, on the point of turning anti-scientific..." So said Snow then, but in this current debate a general lack of scientific understanding is not the critical cause for concern. Moreover, the essentials of the relevant science are relatively easy to communicate. The more significant obstacles to a respectable quality of debate are cultural: the culture of the scientific community, and the culture within which such public controversies arise.

Challenges

Opponents of agricultural biotechnology have several things going for them: principles (such as belief in the virtues of a 'natural' approach) and a capacity to organize and to lobby (an operational coherence undermined when they have government responsibility, as the disarray of the Greens in Germany suggests). Like it or not, they also command a degree of public trust that outweighs that given to government, let alone the biotechnology industry.

In such circumstances, teaching people science does not adequately address the issues. But enhancing public awareness of what science does and doesn't say can at least raise the quality of debate and the wisdom (a quality Snow also explicitly sought in narrowing cultural divides) of the decisions reached. Here, the scientists find themselves at a disadvantage. The gatekeepers in the media (producers, news and features editors) are not the science correspondents, and often have their own sympathies, commercial agendas and incomprehensions which can obstruct the exposure of relevant facts in the midst of headline-grabbing but misleading assertions. Professional scientific bodies have a critical role to play, but in the rapid cycle of daily news they are not geared to supplying a timely and appropriately crafted succession of packaged facts, while governments are reluctant to be perceived to be partisan in coordinating public responses from non-governmental scientists. And industry, too often, is publicly ineffectual.

Here, then, are cultural challenges to scientists and technologists (sometimes hard to distinguish in bioscience) at two levels. One is tactical, where obstacles to spreading the message must be overcome by lobbying that is independent of industry and government, and street-wise with respect to the media and the Internet. Swiss scientists adopted a self-coordinated approach in last year's referendum on the use of genetically modified organisms, and a similar lobbying responsiveness, to other ends, has been achieved by Britain's Save British Science.

There is also the need to bridge divides: to achieve more understanding of objectors' principles at home, and of cultural and societal realities in the developing world, without which novel food technology that may be essential to feed future populations will be insensitively promoted or introduced and fail to be accepted. Many of Snow's original concerns today seem gratifyingly irrelevant in general, but naivety should be avoided where cultural antipathies are very much alive and kicking. □



Biologists make plea to NIH to invest in supercomputer centre

[WASHINGTON] A group of biologists who use supercomputers to model the structure and mechanisms of biological molecules is urging the US National Institutes of Health (NIH) to boost its investment in high-performance computing.

The group, which gathered last week at a meeting in Rockville, Maryland, sponsored by NIH's National Center for Research Resources (NCRR), was virtually unanimous in arguing that NIH is lagging behind leading federal agencies — namely, the Department of Energy and the National Science Foundation (NSF) — in investing in supercomputers.

The suggestions of the group, which will be given to NCRR, include the recommendation that NIH fund a single, central facility that operates a large 'teraflop' machine, the next generation of supercomputer.

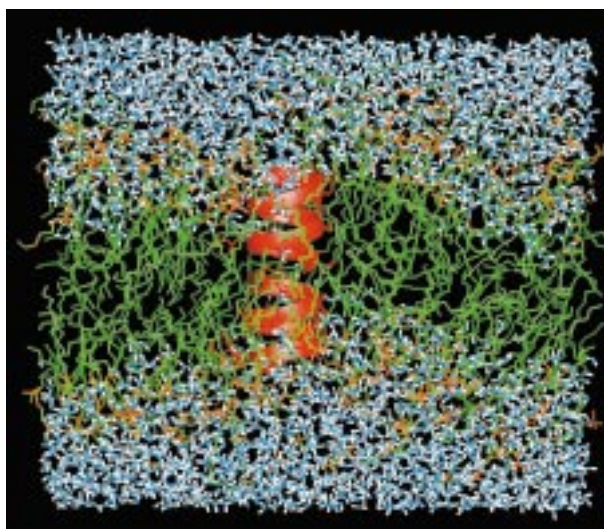
The biologists said that the next generation of 'teraflop machines' — a hundred times more powerful than existing supercomputers — would give qualitative gains, including the possibility of modelling protein-protein recognition and self-assembly. They also spoke of the possibility of studying larger molecular systems, rather than, say, isolated enzymes, leading to a more sophisticated approach to drug discovery.

They also say the machines would allow far longer simulations. As it stands, studies of the changing forces within biological molecules are limited to nanosecond glimpses, preventing tests of theories of molecular structure and function in the real world, where changes take a million times longer.

But "NIH for some reason is just not there," says J. Andrew McCammon, professor of pharmacology and chemistry at the University of California, San Diego. He uses the San Diego Supercomputer Center, one of two major centres funded by the NSF.

"The NIH are not a player in this high-performance computing business, and they really need to be," said Klaus Schulten, who chaired the meeting and heads an NCRR-funded mid-level computer resource at the University of Illinois at Urbana-Champaign. He added that NIH was being asked "with a louder and louder voice" to fund computing centres, but that the biomedical agency has "not addressed the role of computing... as forcefully as some other agencies".

The meeting was organized to list the advances that teraflop machines would allow, to present NIH with a convincing argument for investing the multi-million dollar sums required to make high-performance machines more broadly available.



Complex number: this computer image generated at the National Center for Supercomputing Applications at the University of Illinois shows a model ion channel in a lipid bilayer embedded in a solvent milieu. The image demonstrates the complexity of macromolecular systems. The centre is funded by the National Science Foundation.

The NIH supports only one \$3.9 million, dated supercomputer centre at a National Cancer Institute campus at Frederick, Maryland. Biologists do most of their work at a centre in Pittsburgh, mainly funded by the DoE, and at the NSF-funded centres at San Diego and Urbana.

NSF invests \$70 million annually in its two centres. And the \$366 million government-wide computing initiative recently announced by Vice-President Al Gore allocates only \$6 million to NIH, of which just \$2 million goes to "advanced computing" (see *Nature* **397**, 285; 1999).

The use of the NSF centres by biologists is growing rapidly. Between June 1997 and May 1998, one-third of the time at the NSF supercomputer centres was allocated to (mostly molecular) biologists. This is a 54 per cent increase on the previous year.

"There's a very clear trend that the major

usage and the large projects are swinging over from being physics- and astronomy-driven to being biology-driven," says Eric Jakobsson, a senior research scientist at the NSF-funded National Center for Supercomputing Applications at the University of Illinois at Urbana-Champaign. "In a couple of years the cycles for big users will probably be about 50 per cent biology."

But this does not satisfy biologists, who feel as squeezed as the physicists and astronomers with whom they share the machines, and say that 'cycle drought' — the chronic lack of computer time — should compel NIH to act on their behalf. Overall, requests for time on the leading computer at San Diego exceed that available by a factor of four or five.

Harold Varmus, the NIH director, commissioned a working group on biomedical computing last year to produce a report that will be presented to his advisory committee

Catalonia offers \$16m innovation grants

[BARCELONA] The government of the Spanish autonomous region of Catalonia has launched a US\$16.6 million programme of non-repayable capital grants to encourage the entrepreneurial activities of scientists.

The objective of the scheme, called Network of Centres for Technological Innovation Support, is to finance 100 research teams for the next three years. Each group will receive about \$55,000 a year. Nine research teams have already been approved for funding since the scheme was launched last month.

The scheme is intended to support researchers who have ideas about how their results could be turned into commercial

propositions, but who lack access to the necessary funds. Successful grant applicants will be able to build up the resources needed to offer their knowledge to private industry, or to create their own companies.

The initiative stems from a desire by the government of Catalonia to boost links between the scientific and business communities.

Applicants will have to demonstrate potential commercial interest in their work. They must produce a strategy suggesting how the project might become self-financing within three years. If, after this period, the researcher has not achieved a profitable result, the grant will end.

Xavier Bosch

in June. Part of the group's remit is to investigate "the impediments biologists face in utilizing high-end computing".

Larry Smarr, director of the NSF centre at the University of Illinois and a co-chair of the working group, says he agrees that supercomputers offer a "tremendous opportunity" to biologists. But he says there is a lively debate in his group on how to increase NIH-funded high-performance computing, and no consensus has been reached. The group is looking at the extent to which enlarging existing 'clusters' of personal computers or workstations in research groups could meet the need for advanced computer power.

Also, should NIH invest in a new supercomputer centre, build on the one at Frederick, or develop several smaller centres dedicated to applications such as organ simulations, genomics and the study of biopolymer systems? Or should it add its resources to one of the NSF or energy department centres, where it could profit from cross-pollination with the physical sciences and the existing infrastructure? "My guess is that there will be some sort of combination of solutions," says Smarr.

But, he says, whatever his group recommends, the supercomputer needs of biologists will not be assessed in a vacuum. "Clearly the science that's done with supercomputers can't be done any other way," says Smarr. "But there are vastly more biologists that use the web than use supercomputers. If [NIH] has only so many dollars, then it has to make decisions across the spectrum."

Many of those at last week's meeting argued that the kinds of advances foreseen by advocates of teraflop machines do not fire the imaginations of ordinary biologists. They pointed out that, to sell high-performance computing to NIH, enthusiasts will have to chart a course from these improvements to tangible health benefits.

That's not difficult, says McCammon. He points out that the HIV protease inhibitors now prolonging thousands of lives were developed in part with computational methods from his laboratory.

He says that his lab's work on the neuroenzyme acetylcholinesterase, discovering computationally how the enzyme gets its speed and specificity of action, is laying the groundwork for better drugs for neurological disorders from Alzheimer's disease to glaucoma.

Others said that, regardless of the power of supercomputers or NIH investment, their availability will be partly wasted unless the broader community of biologists becomes better educated in their use.

There is "huge ignorance" among ordinary biologists about how to use computational tools, said Carol Post of Purdue University, Indiana. "The universal problem is not hardware," adds Smarr. "All the biologists [the working group has interviewed] say 'we need more training'."

Meredith Wadman

Mixed funding fortunes for UK research universities

[LONDON] England's top five research universities are — once again — the biggest beneficiaries in this year's allocation of £855 million (US\$1.37 billion) of research funds from the Higher Education Funding Council for England (HEFCE).

In contrast, the small cash increase in research income for most other large research universities represents a drop in real terms. Some, including the universities of Birmingham, Liverpool and Newcastle, even see the cash value of their HEFCE funds fall.

Overall, HEFCE research funds for England's 76 universities and 58 colleges and specialist institutions will rise by 3.5 per cent, one per cent above the rate of inflation. This year, HEFCE has a total university teaching fund of £2.9 billion, including £20 million to support wider participation by students from disadvantaged backgrounds.

The largest increases in research incomes, however, averaging 6.8 per cent, go to the universities of Oxford and Cambridge, Imperial College and University College, London (UCL).

The £60.68 million budget for UCL, currently third in the HEFCE research league table, brings it within reach of the University of Cambridge (£61.3 million), currently in

second place. The University of Oxford (£63.05 million) is ranked first. Imperial College (£54.4 million) is ranked fourth, with King's College London (£35.19 million) fifth.

Next year, UCL may well overtake the University of Cambridge, breaking the long-established Oxbridge duopoly at the top, as Oxford and Cambridge's comparatively large increases for this year partly reflect the transfer of £6 million in undergraduate fees from the Department for Education to HEFCE.

Brian Fender, chief executive of HEFCE, says this year's allocation is a "modest" increase compared to last year's 14.6 per cent increase in research funds. But this, he says, anticipates the extra £600 million for research infrastructure announced last year as part of the Comprehensive Spending Review.

HEFCE funds are one pillar of Britain's dual-support research-funding system, and are used to pay for salaries and some infrastructure. Money for research grants comes from the five research councils.

HEFCE funds are allocated according to the results of the Research Assessment Exercise, which evaluates the quality and volume of university research. The next exercise will be in 2001.

Ehsan Masood

US Nobel winners back stem-cell research

[WASHINGTON] A group of 33 Nobel laureates is urging the US government to stick to its promise to fund research using human embryonic stem cells, despite a ban on funding research on human embryos.

The group wrote to President Bill Clinton and the US Congress last week warning of the "serious negative consequences" of not allowing federally funded scientists to work with the cells (see *Nature* 397, 185; 1999). Such research shows great promise for the development of cell and tissue therapies for diseases from diabetes to Alzheimer's.

But controversy has arisen because some stem cells are derived from embryos left over after fertility treatments. Opponents of abortion argue that federally funded research with these cells breaks the law prohibiting federal funding of human embryo research.

The letter argues that if federally funded scientists are not allowed to proceed it will "bar the majority of the nation's most prominent researchers who are supported by the National Institutes of Health and the National Science Foundation... from engaging in this critical research".

It adds that the use of peer-reviewed federal funds for stem-cell research "is our best assurance that research will be of the highest quality and performed with the greatest dignity and moral responsibility".

The letter was written and circulated by Paul Berg, the Stanford University biochemist who chairs the public policy committee of the American Society for Cell Biology. Its signatories include David Baltimore, president of the California Institute of Technology, J. Michael Bishop, chancellor of the University of California at San Francisco, and Joshua Lederberg, professor emeritus at Rockefeller University in New York.

"We had been concerned about the issue on Capitol Hill and wanted to make it very clear that the scientific community is behind stem-cell research and its funding," says Tim Leshan, director of public policy at the society.

The initiative comes after 70 conservative congressmen and seven senators wrote to Donna Shalala, Secretary of Health and Human Services, to protest at the department's decision to fund the research (see *Nature* 397, 639; 1999).

M. W.

Japan's transplant law 'is too stringent'...

[TOKYO] Japanese patients awaiting organ transplants are calling for a revision of the legislation covering transplantation following Japan's first legal heart, liver and kidney transplants last week. The organs were all taken from one donor.

Without such revision, patients fear that the stringency of the current regulations, which include the need for a family's permission before organs can be taken from a brain-dead donor, will prevent such operations being widely available.

Last week's operations were carried out successfully at several hospitals across Japan, including hospitals at Osaka University and Tohoku University. They came 16 months after legislation was passed allowing the transplantation of organs from brain-dead patients.

While the event has raised hopes for similar future operations, many still think it unlikely that organ transplants will become the medical norm in Japan.

In addition to the general reluctance of the Japanese population to accept the idea of organ transplants, the lack of donors and the strict regulations imposed by the current legislation are seen as serious impediments.

For example, the transplant law requires written consent from both the donor and the donor's family. This means the family can overrule a brain-dead patient's intention to donate the organs.

The law also prevents children from having transplants by requiring that donors are no younger than 15, while children under six cannot be organ recipients.

The high number of patients requiring organs and the low number of potential donors will continue to force people to turn to hospitals overseas, says Yoshi Aranami from Trio Japan, an organization that coordinates organ transplants for Japanese patients.

According to Aranami, 44 Japanese patients, ranging in age from 1 to 55 years, have gone overseas for heart transplants in the past 15 years, while 200 patients have similarly received liver transplants, most of which were successful.

"We just have to explain to patients that going abroad for transplant operations is a more reliable option than staying in Japan," he says.

But with more than 600 patients requiring organ transplants every year, many feel that the legislation must be relaxed to secure more donors. Patients' support groups, such as the Association to Protect Children with Heart Diseases, are calling for the earliest possible revision of the law, instead of the evaluation the government intends next October.



Organs in demand: more than 600 patients require organ transplants each year in Japan.

Hiromu Nonaka, the chief cabinet secretary, has stressed the importance of creating an environment that will make organ transplants more common in Japan. "We must implement policies that give full consideration towards establishing such transplants in Japan," he said at a press conference last week.

Last week's operations were the first to be carried out since Japan's first heart transplant was performed at Sapporo Medical

College in 1968. The operation, commonly known as the 'Wada transplant' after the chief surgeon, attracted wide public criticism when some individuals suggested that the donor may not have been brain-dead when his heart was removed from his body. There was also speculation that the recipient's condition was not severe enough to require a transplant.

The patient's death led to a murder charge against the chief surgeon Juro Wada, although he escaped prosecution through lack of evidence. But the repercussions were long lasting — the incident resulted in a 30-year ban on organ transplants from brain-dead donors until the new transplantation law was passed in 1997 (see *Nature* 387, 835; 1997), and left surgeons and researchers out of touch with advances in transplantation technology.

"We have come a long way to make this heart transplant happen," says Hikaru Matsuda, the chief surgeon at Osaka University Hospital who performed Japan's second heart-transplant operation last week. But he cites the lack of donors and the shortage of skilled surgeons as problems that must be urgently addressed.

Asako Saegusa

... but the pill may be legalized at last

[TOKYO] The Japanese government is expected soon to lift its ban on the contraceptive pill, almost nine years after the first application for its use. The approval of the drug will come almost 40 years after its release in the West.

According to the Central Pharmaceutical Affairs Council, which advises the health minister, approval will probably be recommended during a council meeting in June, with a view to having the drug on the market by the autumn.

The move comes after public criticism of the speed with which the Ministry of Health and Welfare approved Viagra, the male impotence treatment, just six months after the first application.

In contrast, the contraceptive pill has been entangled in a debate over its possible risks to human health. However, as far back as 1985 legalization seemed on the horizon, and the

Japan Obstetrics and Gynaecology Society and the Japan Motherhood Protection Medical Association were asking the Ministry of Health and Welfare to allow clinical tests (see *Nature* 317, 760-761; 1985). Although the government came close to approving it in 1992, they backed down at the last moment amid fears that the release could lead to an increase in AIDS and other sexually transmitted diseases.

The government has since cited concerns about the contraceptive pill being an endocrine disruptor — the man-made chemicals suspected of affecting human reproductive functions — but such claims have so far been brushed aside by scientists.

The government's reluctance to legalize the pill is said to result from a powerful medical lobby that

opposes threats to its lucrative abortion business. According to the ministry, one in five unwanted pregnancies ends in abortion, with more than 340,000 such operations performed each year.

There is also speculation that conservative politicians concerned about the low birth rate in Japan are strongly opposed to approval of the pill.

Approximately 200,000 women are taking the high-dose pill — available in Japan as a treatment for menstrual disorders — for contraceptive purposes.

A spokesman from Schering, the German pharmaceutical company that is one of the nine drug firms awaiting approval of their oral contraceptive, says they are "crossing their fingers" that the health ministry will keep its promise and not reverse its decision at the last moment again.

A

Russia protests at US science sanctions

[MOSCOW] Russian officials have criticized the US decision to impose sanctions against 10 Russian institutions — including three prominent scientific centres — for alleged collaboration with Iran that could aid the development of nuclear missiles.

The three research bodies are the 'Mendeleev' Chemical Technological University (CTU), the Moscow Institute of Aviation (MAI) and the Energy Technical Scientific Research Institute (ETSRI).

Russian prime minister Yevgeny Primakov described the measures as "counter-productive for relations between Russia and the United States, which are of great value to us".

Vladimir Rachmanin, a spokesman for the Ministry of Foreign Affairs, has dismissed US accusations as "groundless" adding that the sanctions "will not go unanswered".

The scientific institutions were identified by Samuel Berger, National Security Advisor to President Bill Clinton's administration, as having broken the nuclear non-proliferation treaty. But Igor Sergeev, Russia's defence minister, says they could not have broken the treaty "because they did not possess full knowledge of [nuclear and launcher] technologies".

Last July, after Iran launched its own rocket, the United States introduced sanctions against seven Russian companies and universities on the grounds that they had supplied Iran, Libya and North Korea with nuclear and rocket technology, materials and equipment of dual — civilian and military — use.



The Russian reaction was relatively mild. The Federal Security Service (FSS) admitted that a few Iranian companies with links to the launcher programme had been operating in Russia, but were no longer doing so.

It was said that the Iranians had tried to order a component of a liquid-type jet engine from the Russian company Trud, claiming it was for pumping gas. According to the FSS, "this attempt was revealed at an early stage and immediately stopped".

Now, however, the FSS says it has failed to find any evidence of dealing in secret documents at the three scientific organizations added to the US blacklist. The institutions deny all the accusations.

Pavel Sarkisov, rector of the CTU, calls the US accusations "absurd". He says: "We have

only one Iranian postgraduate, who is preparing a dissertation on polystyrene synthesis [which] has nothing to do with nuclear research — it is used in the building industry, and engineering." Sarkisov says the sanctions will have no effect on the CTU, as it has no contracts with the United States.

But the CTU has a prominent research programme in nuclear-waste reprocessing. According to the ecologist Alexei Yablokov, former adviser to President Boris Yeltsin on nature conservation and public health, "CTU is engaged in developing the chemical component for the rocket fuel".

This could explain the Clinton administration's measures, described in a statement as intended "to cease the transfer of sensitive Russian technology to Iran".

Alexander Matvienko, rector of the MAI, admits that 28 Iranians are studying at his university. But he insists that they have no access to secret materials.

US concern seems to stem from the MAI's collaborative research agreement with Iran. The university will be badly hit by the sanctions, as it has contracts worth \$500 million with US companies, while its staff — like those at the CTU — will no longer be able to make US lecture tours.

ETSRI is one of the leading institutes of the atomic energy ministry, and ministry head Oleg Adamov was ETSRI's director for more than a decade. "We are much more concerned than the United States with the possibility that Iran could have nuclear weapons and the rockets to deliver them, as Iran is our neighbour; only the Caspian sea divides us," he says. "We are therefore being very cautious not to promote Iran's rocket and nuclear weapons programmes."

Last spring, at a meeting with Reza Agk-hazadeh, Iranian vice-president and president of the Iranian organization for atomic energy, Adamov said that "all co-operation with Iran in nuclear technologies is within the frame of the non-proliferation treaty, and in accordance with the dual technologies list".

ETSRI will be worst hit by the sanctions. It has received many grants from the International Scientific Technical Centre set up with US help to dissuade Russian scientists with knowledge of 'sensitive technologies' from leaving the country to join 'unstable regimes'. The loss of this money could be more significant than the \$800 million contract that Adamov signed with Iran for the construction of an atomic power station.

The United States has also threatened to freeze its contacts with Russian space programmes if Russia does not stop supplying Iran with rocket technology. As a result, the Russian space agency might not receive the \$1 billion it is expecting for launching US satellites with its Proton rocket. Carl Levitin

Los Alamos secrets 'were leaked to China'

[WASHINGTON] A major security breach involving a Chinese-American computer scientist working at the Los Alamos nuclear weapons laboratory in New Mexico has enabled China to copy the United States' most important recent nuclear warhead, according to congressional investigators and Department of Energy officials.

The New York Times reported last weekend that the scientist failed a polygraph test last month and has been moved from nuclear weapons work, but not charged with any offence. The scientist, Wen Ho Lee, fell under suspicion in 1997, but was allowed to remain in a sensitive position at the laboratory while an FBI investigation continued.

On Monday (8 March) the energy department announced that he had been dismissed.

Some department officials and investigators from the Intelligence Committee of the House of Representatives believe that the alleged espionage enabled China to develop and test a warhead similar to the W88, the compact nuclear warhead used in the US Trident II submarines. Data from Chinese weapons tests led US officials to suspect espionage at Los Alamos, where FBI investigations focused on one suspect.

But other intelligence officials have questioned the importance of the alleged espionage. They argue that China could have developed

a warhead with similar characteristics to the W88 by itself, or with information from Russia. China has already denied that espionage took place.

In a statement this week, Edward Curran, head of counter-intelligence at the energy department, said that "the extent to which these disclosures may have helped part of the Chinese weapons programme is unclear". But he confirmed that steps had been taken to increase security.

The allegations will further intensify pressure to tighten security at the national laboratories. After criticism from Congress, the laboratories have restored full checks on visitors from certain countries (see *Nature* 395, 627; 1998). Colin Macilwain

Top physics labs 'must bury the hatchet'

[WASHINGTON] Michael Witherell, a physicist at the University of California at Santa Barbara, has been appointed director of Fermilab, the largest high-energy physics laboratory in the United States.

Witherell, an experimentalist and member of the National Academy of Sciences who pioneered the use of more powerful detectors and high-speed data acquisition systems in particle physics, will replace John Peoples as director of the laboratory at a critical time (see *Nature* 394, 611; 1998).

Within six years the Large Hadron Collider will open at the European Laboratory for Particle Physics (CERN) in Geneva, and Fermilab's main injector, its largest experimental facility, will lose its place at the high-energy frontier of particle physics.

Together with Jonathan Dorfman, another young physicist recently named as the next director of the Stanford Linear Accelerator Center (SLAC) in California, Witherell faces the task of reinvigorating a US high-energy physics community still recovering from the 1993 cancellation of the Superconducting Super Collider (see *Nature* 365, 773; 1993).

"Fermilab has a terrific physics plan for the next five or six years," Witherell said in an interview. "What happens after that is the big issue, not just for Fermilab, but for all of high-energy physics in the United States."

Witherell also thinks it is time SLAC and Fermilab, the primary centres of the disci-



Witherell: challenges ahead for particle physics.

SLAC from Burt Richter on 1 September.

Although Witherell lacks experience in managing a major facility, having spent his entire career with university teams at Department of Energy facilities such as Fermilab, he is familiar enough with the politics of the laboratories: for the past three years he has chaired the High Energy Physics Advisory Panel, advising the energy department on its \$700-million particle-physics programme.

Witherell won't talk about Fermilab's long-term direction, saying only that both front-runner proposals for future machines

plaine in the United States, buried their traditionally fierce rivalry. "I think it is a great opportunity that Jonathan Dorfman and I will be coming in at the same time."

SLAC and Fermilab "need to work together", he says, and if they can't, "we shouldn't be directors". Witherell takes over at Fermilab on 1 July, and Dorfman assumes the directorship of

there — a new lepton collider and a muon collider — "need to be looked at very seriously".

But the sensitive inter-laboratory relations the new director can expect to deal with were well illustrated last week with Fermilab's announcement that a team there had demonstrated direct CP violation — the asymmetry between matter and antimatter — more emphatically than before. The result will be published shortly in *Physical Review Letters*.

Fermilab's announcement drew an unusually direct public riposte from Konrad Kleinknecht, professor of physics at the University of Mainz in Germany. He issued a statement saying that a similar result had been found 11 years ago at CERN, and was subsequently "contested by an experimental group at Fermilab".

The Fermilab result "is a brilliant confirmation of the earlier observation at CERN, and deserves credit for that", Kleinknecht's statement said.

Bruce Winstein of the University of Chicago, spokesman for the Fermilab experiment, said the laboratory "had bent over backwards to be gracious to CERN", whose work was indeed discussed and credited in the Fermilab announcement.

But Winstein admitted that he hadn't noticed the headline on the Fermilab press release — "Fermilab physicists find new matter-antimatter asymmetry" — when he approved it.

Colin Macilwain

The search for missing mass finds funds for UK researchers

[LONDON] British efforts to discover the missing mass of the Universe are to receive a cash injection of £5.2 million (US\$8.3 million) over the next four years in an attempt to consolidate Britain's position among the top research groups in the field.

Research into dark matter, which makes up 90 per cent of the mass of our Galaxy, has the personal backing of Ian Halliday, chief executive of the Particle Physics and Astronomy Research Council which is giving the money. Halliday believes it to be a field in which Britain could win a physics Nobel prize (see *Nature* 392, 217; 1998).

Neil Spooner, a spokesman for the British dark-matter project, a collaboration between Imperial College, London, the University of Sheffield and the Central Laboratory of the Research Councils, says the 20 per cent increase in funds is "excellent news" — although he acknowledges that a 60 per cent increase had been requested.

More funds, however, will be one of the outcomes if the group comes up with positive results. It has also applied to the government's £600 million Joint Infrastructure Fund for £3.8 million for new laboratory facilities.

British efforts to detect weakly interacting massive particles (WIMPs), believed to be a constituent of dark matter, are housed in Yorkshire, down a one-kilometre-deep disused salt-mine where background radiation levels are low; one of the biggest challenges for dark-matter researchers is in distinguishing WIMPs from natural background radiation.

The new funds will be used to build several detectors, including a 50-kilogram sodium-iodide detector, and two liquid-xenon detectors known as 'Zeplin'. The group also plans to develop a xenon-gas-based detector called 'Drift' to measure the direction of dark-matter particles.

Despite the cash injection, Spooner says, the British group remains less well funded than its two main competitors, a group at the University of Rome and one based on a collaboration between researchers at the University of California, Berkeley, and Stanford University.

None of the groups has so far claimed to have discovered dark matter. The US group has only recently overcome various technical problems with its cryogenic detectors, and is

expecting to start gathering data this year.

The Rome group, which uses a 100-kilogram sodium-iodide detector, has recorded an apparent seasonal variation in its radiation counts, with more counts in summer than in winter, which is one indication of a dark-matter signal. But Peter Smith of the Rutherford Appleton Laboratory, the founder of the British group, says more research is needed before the variation is confirmed.

While welcoming the research council funds, one leading particle physicist believes that research grants should be driven by exciting science rather than Nobel prizes, which, he thinks may have outlived their relevance to the way science works today. "Science Nobel prizes are awarded to individuals. But much research today, particularly in particle physics, involves extensive collaborations," he says.

The difficulty of identifying suitable recipients, he adds, would be apparent if the UK group were to win the physics prize for discovering dark matter, as this group includes researchers from the United States, Russia and Italy.

Ehsan Masood

Food scientist in GMO row defends 'premature' warning

[LONDON] The UK researcher at the centre of last month's storm over genetically modified foods says he has few regrets about publicly discussing unpublished research which, he claims, indicate that rats fed genetically modified potatoes suffered ill health.

Giving evidence to the House of Commons Select Committee on Science and Technology on Monday (8 March), Arpad Pusztai said he was concerned that genetically modified foods were being introduced without adequate safety tests. Pusztai was formerly a senior researcher at the Rowett Research Institute in Aberdeen.



Pusztai: success in stimulating debate.

He told the committee that preliminary results from his experiments were causing him concern, and that he had to take action. "What I have achieved is that we are all sitting here and talking about it."

Pusztai's call for tougher regulation of genetically modified products was backed by his former boss, Philip James, director of the institute, in evidence to the committee.

But the two men offered very different versions of the events of last August, when Pusztai's interview in a television documentary led directly to the recent panic over genetically modified foods.

Pusztai said on television that he had discovered that rats fed with potatoes modified to produce an insecticidal lectin called GNA suffered damaged immune systems and stunted growth. He said James spoke to his wife by telephone after the broadcast "to congratulate me on how well I handled the interview".

But the following day, Pusztai discovered that the Rowett institute had issued a press release claiming that he had inadvertently revealed the results of feeding studies with a different lectin, Con A, which is not used in food as its toxicity is well known.

Although Pusztai denies mixing his data, he says he was suspended and his data confiscated, and claims that staff were warned not to talk to him. James told the committee that Pusztai had not only seen the press release, but had rewritten part of it. "Pusztai had presented information that turned out to be untrue. There was confusion in his group, and his collaborators were outraged," said James.

An audit committee subsequently set up by Rowett said Pusztai's data on the effects of feeding rats with GNA modified potatoes did not support his claims.

Ehsan Masood

US State Department gets cold feet about cold fusion

[WASHINGTON] A meeting that was to have been held at the US State Department next month to highlight research on unconventional energy sources seems likely to be scaled down or cancelled because of doubts about the credibility of the research.

The First International Conference on Free Energy was to have taken place on 29 and 30 April at the State Department auditorium. The conference is sponsored by the Integrity Research Institute (IRI), a Washington-based group that promotes exotic energy concepts ranging from 'magnet power' to 'assisted nuclear reactions' — one description of cold fusion.

The department agreed more than a year ago to host the meeting as part of a programme of lunchtime talks meant to encourage dissenting views on foreign policy issues. IRI president Thomas Valone says this would be an appropriate venue because many of the 'free energy' concepts to be discussed would have a bearing on US compliance with the Kyoto climate-change treaty.

But the meeting has grown to a two-day conference, with more than a dozen speakers, and hundreds of people attending.

Meanwhile, responsibility for coordinating the Open Forums programme has changed hands twice at the State Department. According to Valone, the most recent individual to take on the job, Cora Foley of the department's Bureau of Intelligence and Research, questioned whether the department should support the conference shortly after assuming her post a few weeks ago.

Sources say Foley and other department officials have received e-mails and calls from mainstream scientists asking why the department is lending its name to a meeting they argue has little scientific merit.

After a meeting between Foley, Valone and others on Monday (8 March), the department remained undecided as to whether it will cancel or curtail its involvement in the conference. One source said the organizers were considering moving it to a hotel.

Valone, who is also a patent examiner with the US Patent and Trademark Office, found himself in hot water with his own agency last year when he sent out an Internet notice to "all able-bodied infinite energy technologists", inviting them to apply for examiner jobs at the agency and thereby "infiltrate" the office to encourage openness to unconventional ideas. Valone says he was chastised by the patent office, and admits he made a mistake in sending out the notice.

Robert Park of the American Physical Society, who often ridicules cold fusion and similar concepts in a weekly electronic newsletter on science policy, says he finds nothing wrong with a conference on free energy. "The problem is giving some sort of State Department imprimatur to it that makes it look like the federal government is taking this seriously," he says. "That's what these people are lusting for."

In fact, says Valone, one Australian researcher who planned to attend has used the State Department's involvement to try to enlist the participation of his government. Valone had hoped the department's endorsement "might have some effect worldwide".

The Department of Energy refused to act as a co-sponsor. One energy department researcher who still planned to attend, David Goodwin of the Office of High Energy and Nuclear Physics, says the agency felt "some apprehension about the cold fusion speakers", and agrees "it would be awkward for us" to sponsor the meeting.

Tony Reichhardt

£150m tax break for research in UK budget

[LONDON] The British government has fulfilled earlier promises to provide tax relief for small high-technology companies. The move was announced in the government's budget on Tuesday (9 March).

Gordon Brown, the Chancellor of the Exchequer, Britain's finance minister, said that that the budget was intended to help turn Britain "into a modern knowledge-based economy"; in particular by "championing the needs of small industry".

A tax credit worth a total of £150 million will be available for small high-technology companies, allowing them, according to Brown, to "underwrite 30 per cent of their total research and development costs".

A sum of £325 million is being set aside to allow small and medium size companies to set off capital investments against tax. And a £1.7 billion 'computers for all' initiative is being launched to promote the use of information technology.

Brown announced that an additional £100 million would be allocated to the science base to improve university laboratories and equipment, and that the funds allocated to the government's University Challenge scheme will be increased by 30 per cent.

He also announced measures to reduce Britain's greenhouse gas emissions by three million tonnes.

E. M.

Africa splits over bar to patents on plants

[LONDON] An Africa-wide consensus to restrict the patenting of plant varieties by overseas companies appears to be in disarray following a decision by 15 representatives of French-speaking countries to break ranks.

These countries have agreed instead to recommend the latest version of the International Convention for the Protection of New Varieties of Plants, known as the UPOV convention.

The decision was made two weeks ago at a meeting of patent-office officials from member states of the Organisation Africaine de la Propriété Intellectuelle (OAPI), the regional patent office for francophone Africa. The meeting was held in the Central African Republic.

Accession to the UPOV convention, which grants plant breeders intellectual-property rights over the commercialization of products such as seed, was also due to be discussed this week by 14 English-speaking African countries at a meeting organized by their regional patent office, the African Regional Industrial Property Organization (ARIPO), in Zimbabwe.

Zimbabwe and Kenya, which have a large community of plant breeders, are leading the calls for ARIPO states to ratify the UPOV convention. But environmentalist organizations such as the Canada-based Rural Advancement Foundation International are urging governments to stay out, on the grounds that ratification will prevent small farmers from saving seeds for re-use.

UPOV officials, however, point out that the convention allows subsistence farmers — those who grow crops to feed their families,

but not to sell — and state-funded scientific organizations to save seeds for replanting.

These developments will cast a shadow over an agreement reached last June by the heads of government of the 53-member Organization of African Unity (OAU) to restrict patents on plant varieties until an Africa-wide alternative system to patents has been developed.

This system, which is expected to be published in draft form later this month, will aim to divide the intellectual-property rights of new plant forms between plant breeders and indigenous communities that might have contributed to early varieties.

Johnson Ekpere, executive secretary of the Scientific, Technical and Research Commission of the OAU, says the decision by the organization's heads of state still stands. He says it was reached at a meeting of heads of government in Lusaka, Zambia, attended mainly by representatives of foreign ministries.

But Ekpere admits that details of this decision have not filtered down to science ministries and patent offices. "This is a case of the right hand not knowing what the left hand is doing," he says.

He describes as "unlikely" any attempts to ratify the UPOV convention in an African parliament. Mzondi Haviland Chirambo, director-general of ARIPO, agrees, believing that ARIPO member states are unlikely to follow the lead set by OAPI countries.

Both Chirambo and Ekpere believe that African countries will want to delay legislation until the outcome of a review of the relationship between TRIPS — a World Trade Organization agreement on intellectual-



Cameroon has stopped the US National Cancer Institute from studying *Ancistrocladus korupensis*, a plant with anti-HIV potential.

property rights — the UN biodiversity convention and the UPOV convention.

All member countries of the World Trade Organization are required to frame their patent laws around TRIPS, which says that countries that prohibit the patenting of plant varieties must provide an alternative system of protecting the intellectual-property rights of plant breeders.

But the biodiversity convention is interpreted by some as suggesting that the benefits — including commercial benefits — from biodiversity should not be restricted to plant breeders, but should include those who may have contributed to a discovery in the past.

Ehsan Masood

Call for research and education to tackle 'environmental injustice'

[WASHINGTON] Despite a lack of hard evidence that poor and minority communities face a disproportionate share of environmental health risks, policy makers should take 'environmental justice' seriously and step up their research. So concludes a report from the Institute of Medicine, part of the US National Academy of Sciences.

The study, chaired by James Gavin, senior scientific officer at the Howard Hughes Medical Institute in Chevy Chase, Maryland, was commissioned by four federal agencies involved in environmental health: the National Institutes of Health, the Department of Energy, the Environmental Protection Agency, and the Centers for Disease Control and Prevention.

All have struggled with the issue of environmental justice, which, nine years after the first government-sponsored conference on the subject, remains poorly understood and documented.

The committee could find little research in environmental and occupational medicine that included data specifically on what it calls "communities of concern". And teasing out the effects of environmental stress factors, such as airborne particulate matter, from other health threats facing poor and minority communities is extremely difficult.

"Adequate data are not available in most instances to examine the relationships among the environmental, racial, ethnic, and other socioeconomic determinants of adverse health outcomes," wrote the panel.

As well as reviewing the scientific literature, committee members visited low-income and minority communities in Chicago; New Orleans; El Paso, Texas; Nogales, Arizona; and Hanford, Washington.

Based on the visits and the available science, the panel found that communities of concern "experience a certain type of

double jeopardy". They are more exposed to environmental stress factors, but lack the knowledge and political influence that might lead to change.

Policy makers should therefore concentrate not only on filling gaps in the scientific data, it concludes, but also on improving environmental education and community involvement. The panel warns against rash action based on incomplete information, for example closing an industrial plant that is doing no real harm and may be providing important jobs.

Further research would bring the same benefits to poor communities as to everyone else: "Environmental health sciences research can contribute to environmental justice most effectively by identifying hazards to human health, evaluating the adverse health effects, and developing interventions to reduce or prevent risks for all members of society."

Tony Reichhardt

Call to double US funds for research on cancer among minorities

[WASHINGTON] The US Intercultural Cancer Council (ICC) has called for a \$300 million increase in funding for research by the National Institutes of Health (NIH) to address the higher incidence and mortality rates of some cancers in ethnic minorities and the poor.

"Anything short of a 100 per cent [\$300 million] increase cannot begin to address the tragic disparities in cancer incidence and mortality in these communities," said Lovell Jones, a co-chair of ICC and director of experimental gynaecology and endocrinology at the University of Texas M. D. Anderson Cancer Center, at a meeting in Washington last week.

The group's demand follows the release of an Institute of Medicine report calling for the NIH to do more research on cancer in minorities and the poor (see *Nature* **397**, 283; 1999).

Separately, NIH director Harold Varmus has warned the House of Representatives subcommittee that funds the institutes against giving NIH's Office of Research on Minority Health grant-giving power. In response to a question from Jesse Jackson

(Democrat, Illinois), Varmus argued that the NIH should not "segregate" minority health research.

NASA fears the worst as satellite goes awry

[WASHINGTON] Engineers at the US space agency NASA are uncertain whether they can salvage the \$46 million Wide Field Infrared Explorer satellite, which went into a violent spin hours after its launch last Thursday (4 March). Ground controllers believe the satellite, intended to conduct a four-month survey of the infrared sky, is venting the frozen hydrogen that cools its infrared telescope and detectors and gives them their sensitivity.

The mission, the first in NASA's Origins programme to address questions ranging from galaxy formation to the origin of life, was designed to study ultraluminous galaxies, starburst galaxies, brown dwarfs and other infrared sources.

Israelis join Europe's Framework programme

[MUNICH] Israel and the European Commission last week signed an agreement allowing Israel to participate in the non-nuclear activities of the commission's fifth

Framework research and development programme. In return, Europe's researchers will gain access to Israeli research programmes in similar fields. Israel's associate membership of Framework was agreed after political objections to its involvement had been lifted (see *Nature* **397**, 289; 1999).

Ruth Arnon, professor of immunology at the Weizmann Institute in Rehovot, says she is relieved that Israeli scientists will have access to the Framework programme from the start — protracted negotiations meant they were only able to join the fourth programme halfway through. But she points out that Israel continues to be excluded from the European Science Foundation.

French network focuses on microtechnologies

[PARIS] The French government announced last week that it is setting up a national network of research in micro- and nano-technologies. The move stems from the decision, announced by prime minister Lionel Jospin last summer, that technology research networks would be created to support collaboration between public research institutions and private enterprise in priority areas.

The network will be coordinated from

Grenoble, and will involve researchers from a range of publicly-funded bodies — including universities, the National Centre for Scientific Research and the Atomic Energy Commission — as well as large and small private companies.

A total of FF55 million (US\$9 million) will be available this year for new projects, which will be chosen by a selection committee of industrial and university researchers.

Further networks due to be launched later this year will be focused on food production, health, information technology and energy.

Middle East could go thirsty, warns report

[LONDON] Supplies of high-quality fresh water to countries in the Middle East could run out unless Israel, Jordan and the Palestinian Authority work together to preserve aquatic ecosystems, according to a joint study by the region's research councils together with the US National Research Council.

The study says that regional cooperation is essential for scientists who need to “openly exchange research on water science” in order to effectively monitor water availability and use. “Because of historical and political constraints, data on population, hydrology, and economic

conditions often have been difficult to collect and analyse in a consistent manner,” says the report.

European centre for mouse genetics opens

[ROME] The Italian president, Oscar Luigi Scalfaro, this week opened the Adriano Buzzati Traverso campus at Monterotondo near Rome, an international centre specializing in mouse genetics. It houses the European Mouse Mutant Archive, the Mouse Genetic Programme of the European Molecular Biology Laboratory, and the National Research Council Institute for Cell Biology.

Courses in various aspects of mouse genetics will begin in November, organized in collaboration with the Jackson Laboratory in the United States, which hosts the US mouse mutant archives. The first course will include cryopreservation of embryos, sperm and ovaries.

£3m UK fund to boost creativity in physics

[LONDON] Britain's Particle Physics and Astronomy Research Council has set up a £3 million (US\$4.8 million) fund for innovative research projects in astronomy,

planetary science and particle physics. According to Ian Halliday, chief executive of the research council, the fund is designed to “encourage originality and creativity” and to “break out of the envelope of current research programmes”.

Halliday says the council is particularly keen to encourage high-quality proposals from inexperienced researchers or those who are employed on short-term contracts. Awards will last up to three years. The closing date for applications is 30 June.

Japan backs innovatory small businesses

[TOKYO] Japan's Ministry of International Trade and Industry last week unveiled a plan to support venture businesses through a scheme modelled on the Small Business Innovation Research programme in the United States.

The programme is backed by seven science-related ministries, and will provide funding to small businesses carrying out innovative research, particularly in biotechnology and information technology, with commercial potential. The initiative is expected to receive ¥50 billion (US\$410 million) from the government's third supplementary budget in the autumn.

It's time to 'out' the selfish researchers

Sir — I recently had considerable difficulty in obtaining a clone from the authors of a paper published in *Nature*. The reason the authors gave for their initial refusal to supply the clone was because they did not wish to form new collaborations and that they had links to pharmaceutical companies. This prompts me to make two points.

First, it is one of the conditions of publication in *Nature* (and many other scientific journals) that "authors are required to make materials and methods freely available to academic researchers for their own use", including DNA sequences. This policy is stated clearly in the guide for authors submitting papers to *Nature*. What can be done if the authors fail to abide by these conditions after publication? An article cannot be withdrawn in retrospect, but it should be possible for a journal to refuse to publish further papers from such authors without firm reassurances that the conditions are adhered to in future. However, this would

be difficult for a journal to police, and in itself raises ethical questions.

Perhaps it is for those researchers who come across such restrictive practices to 'out' these groups in the hope that others will take note and embarrass them by peer-group pressure. All researchers can understand the concern of overlapping research and the pressures of having competition in their own area of interest. But this tends to generate healthy debate and discussion. I suspect that most researchers would rather be working in a competitive area than a backwater with no competition.

Second, I am concerned that, because of collaborative work with an academic institute, a company can inhibit the ability of other academics to work in this area by restricting the distribution of material, and hence maintain a degree of control over the subject. All pharmaceutical companies are secretive about their research to try to gain an advantage over their competitors and to

protect patentable advances. The pharmaceutical industry is a repository of knowledge that may never reach the public domain: for example, a previous correspondent has complained about the lack of published data on lexipafant hindering his ability to defend his thesis (*Nature* **395**, 431; 1998).

But, although I am not against members of the pharmaceutical industry trying to protect their research data, I am against them hindering interest-driven research elsewhere. This is against the spirit in which many scientists conduct their work and this type of practice should not be allowed to creep into public institutions. Academic research should be for the benefit of all and we should pursue our goals with the intention of disseminating that knowledge freely.

Noel C. Harris

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PMs and policemen are getting younger

Sir — Older people commonly complain that they do not enjoy the respect of younger generations. The complaint is the more bitter because they feel they would have been respected in old age had they been born a century earlier. In the absence of any data from the eighteenth and nineteenth centuries to test the validity of this complaint, I decided to analyse the age at which British prime ministers and US presidents first took office. I reasoned that, by and large, voters would elect a leader from an age group they respected.

The results (Fig. 1) reveal a striking contrast between the two countries. British prime ministers became progressively older through the eighteenth and nineteenth centuries. In the twentieth century this trend is reversed. In the United States, there is no significant pattern. There were several quite old presidents at the outset, as four Founding Fathers took turns in the job. In the twentieth century, there have been some notably old presidents (Ronald Reagan and George Bush) and some notably young ones (John F. Kennedy and Bill Clinton).

The British changes could have some connection with changes in the demographic profile of voters. Increases in life expectancy will make voters older on average while extensions in the franchise may have the reverse effect. The former is likely to be the stronger effect. However, I

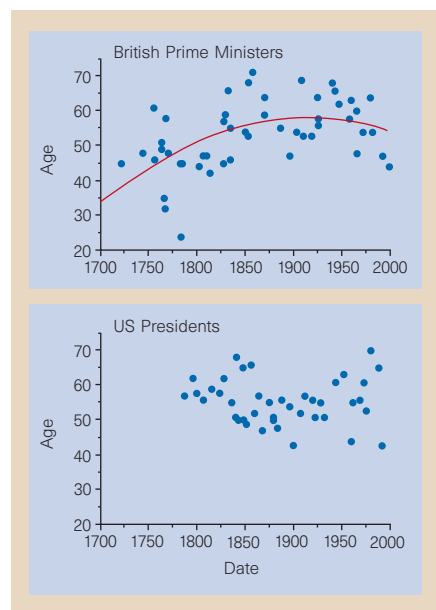


Figure 1 The age at which British prime ministers and US presidents first came to power plotted against the year in which they assumed office. The US data show no significant trend but the British pattern is highly significant ($P < 0.001$), and the line shows the fitted second order polynomial.

wish to offer an alternative perspective.

Biologists commonly argue that contests for mates and/or resources are often resolved by honest signals of quality¹. Since such signals are costly to display, they cannot be displayed by low-quality cheats and therefore they reliably signal the high quality of the signaller. Could such arguments apply to the British data?

Two hundred years ago, the ability to survive beyond 50 indicated a genetic constitution able to cope with an environment where disease was widespread. Only in this century did life expectancy advance beyond 50 in developed countries². Today, UK life expectancy is over 70, and survival to that age has ceased to indicate anything much about genetic constitution.

In Victorian times, prime ministers were drawn from the élite of society and proved their ability to operate in that stable milieu. In today's more rapidly changing society, there is a case to be made that the ideal prime minister is one who has risen to prominence quickly because he or she shows the necessary flexibility to lead the nation in a time of transformation.

I suggest that British voters give the top job to somebody whose attributes are honest signals of ability in the society of the day. Prime ministers became older as life expectancy increased. But the pattern reversed this century when voters realized that an elderly member of the élite was no longer a likely possessor of useful physical or social qualities. At a less elevated level, this may sadly mean that today's older people are correct to grumble about lack of respect. What is less evident is why US voters are not following the British pattern.

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Moving protein heads for breakdown

Martin Scheffner

To be broken down, p27^{Kip1} — a protein that regulates the cell cycle — must be exported from the nucleus. This unexpected step could turn out to be a general way to regulate the turnover of many nuclear proteins.

The orderly transit of cells through the cell cycle requires a delicate balance between positive and negative regulatory factors. Any alteration in this balance can result in abnormal cell proliferation, which may contribute to cancer. Proteins whose activity is required only at certain stages of the cell cycle can be inactivated by selective protein turnover, and target proteins are often flagged for destruction by the addition of a phosphate group (a process known as phosphorylation). These proteins are then further modified by linkage to a small polypeptide called ubiquitin, and finally digested by proteasomes — large, hollow cylinders containing protein-digesting enzymes which bind to the ubiquitinated proteins and finish them off.

On page 160 of this issue, Tomoda *et al.*¹ report that for one protein, p27^{Kip1}, proteasome-mediated degradation requires both phosphorylation *and* export from the nucleus. This export step, in turn, seems to be controlled by the proteasome. So, the precise localization of p27^{Kip1} within the cell determines its metabolic fate, indicating that protein breakdown is regulated not only in time, but also in space.

The ubiquitin/proteasome system is important for turnover of many regulatory proteins in the cell². One of the fundamental questions about selective protein degradation is how individual substrate proteins are specifically targeted at any given time. This problem is particularly obvious with proteins that are broken down only at distinct stages of the cell cycle. In a simplistic view, the ubiquitin/proteasome system works in two steps. First, substrate proteins are modified by the covalent attachment of ubiquitin molecules. These 'polyubiquitinated' proteins are then recognized by the so-called 26S proteasome and degraded.

All of this implies that protein degradation can be timed by regulating the interaction between the target protein and components of the ubiquitin system that recognize it. Indeed, in some cases, the part of the ubiquitin-conjugation system that recognizes the target is active only at distinct phases of the

cell cycle. In other cases, the recognition component may be permanently switched on, but the substrate protein is recognized only on phosphorylation — which, in turn, occurs only at a distinct stage of the cell cycle².

This model — that phosphorylation triggers degradation — has been put forward in recent years as a mechanism for the specific breakdown of p27^{Kip1}. The p27^{Kip1} protein normally acts at a particular stage of the cell cycle to inhibit another group of cell-cycle proteins. Several studies indicate that p27^{Kip1} is a substrate of the ubiquitin/proteasome system, and that its breakdown depends on its phosphorylation status^{3–5}. But Tomoda and colleagues¹ now provide good evidence that this is only half the story. Using a yeast two-hybrid screen to identify proteins that

interact with p27^{Kip1}, they fished out a mouse protein termed Jab1 (ref. 6). When the authors characterized this interaction between Jab1 and p27^{Kip1}, they found that increasing the levels of Jab1 causes increased breakdown of p27^{Kip1}. Moreover, Jab1 can overcome the blocking effect of p27^{Kip1} on the other cell-cycle proteins (and, hence, on the cell cycle itself). Similarly, overproduction of Jab1 means that cultured cells are less sensitive to serum starvation (which is another way of blocking the cell cycle, and probably acts via p27^{Kip1}).

These results clearly indicate that Jab1 controls the activity of p27^{Kip1} by facilitating its degradation. But how — and where — it does this is surprising. First, Tomoda *et al.* show that binding of Jab1 directs the movement of p27^{Kip1} from the nucleus to the cytoplasm. This Jab1-dependent relocalization is necessary for breakdown of p27^{Kip1}, as a mutant p27^{Kip1}, which cannot bind to Jab1, is neither exported nor destroyed. Likewise, p27^{Kip1} is not broken down in the presence of leptomycin B, a drug that interferes with the export of proteins from the nucleus. Second, the presence of proteasome inhibitors interferes not only with degradation of p27^{Kip1}, but also with its movement from the nucleus to the cytoplasm. At first glance, this may seem puzzling. But there's an easy explanation if we consider that the proteasome may control the half-life of either a factor that specifically interferes with export of p27^{Kip1}, or a more general inhibitor of nuclear export.

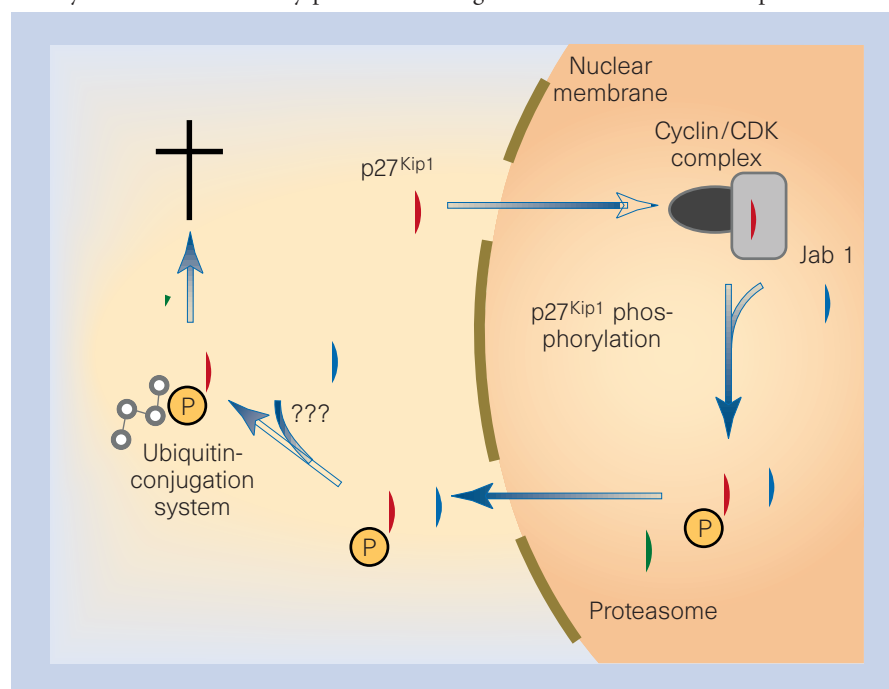


Figure 1 Suggested pathway for turnover of p27^{Kip1}. Because p27^{Kip1} is required only at certain stages of the cell cycle, its activity is tightly controlled by several mechanisms, among them protein breakdown. This has previously been shown to be triggered by phosphorylation of p27^{Kip1}, leading to binding of the polypeptide ubiquitin and subsequent digestion by the proteasome. But Tomoda *et al.*¹ have now identified a factor called Jab1, which binds to p27^{Kip1} and shuttles it from the nucleus to the cytoplasm. The export of p27^{Kip1} is controlled in a proteasome-dependent manner and, in addition to phosphorylation, is required for p27^{Kip1} degradation by the ubiquitin/proteasome system.

So, from the available data, we can come up with a model for breakdown of $p27^{Kip1}$ (Fig. 1). After it has been produced, $p27^{Kip1}$ is transported into the nucleus where it interacts with, and inhibits, its target cell-cycle proteins. Then, when it must be inactivated, $p27^{Kip1}$ is phosphorylated and, on binding Jab1, it is shuttled from the nucleus to the cytoplasm. Finally, $p27^{Kip1}$ is recognized by the ubiquitin/proteasome system in the cytoplasm and degraded.

But this model raises several questions. For instance, because the 26S proteasome seems to be present in both the nucleus and the cytoplasm, why is $p27^{Kip1}$ degraded only in the cytoplasm? Why not in the nucleus? Does this indicate that the parts of the ubiquitin-conjugation system that recognize $p27^{Kip1}$ are found only in the cytoplasm? Similarly, what is the exact role of Jab1 in $p27^{Kip1}$ degradation? Perhaps Jab1 simply shuttles $p27^{Kip1}$ from the nucleus to the cytoplasm. Alternatively, because Jab1 is present in mammalian COP9 complexes⁷ (which seem to contain enzymes that add phosphate groups), it may also be involved in phosphorylating $p27^{Kip1}$. Or, even more excitingly, perhaps Jab1 itself physically interacts with components of the ubiquitin/proteasome system.

The finding that degradation of $p27^{Kip1}$ requires nuclear export indicates that its activity is regulated at this transport step. In this case, the ubiquitin/proteasome system

does not act as a true regulator, but rather as a terminator by irreversibly inactivating $p27^{Kip1}$. Other reports indicate that the degradation of two other cell-regulatory proteins, p53 and cyclin D (refs 8,9), also involves their export from the nucleus. So, the link between nuclear export and degradation may emerge as a common scheme for turnover of nuclear proteins. It will be interesting to see whether, in these cases, nuclear export is also regulated by the proteasome. In any event, the findings of Tomoda *et al.*¹ should also serve as a cautionary note — that just because a protein is stabilized by proteasome inhibitors, it does not necessarily mean that this protein is a direct substrate of the proteasome. □

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the magnetic coupling. The coupling can align the magnetization of the two magnetic layers either in the same direction or opposite to each other, depending on the thickness of the spacer. If the alignment is opposite, then a small magnetic field can realign the magnetization to be in the same direction. This realignment in the presence of an applied magnetic field can be accompanied by a dramatic change in the electrical resistance of the structure.

This magnetoresistance has been dubbed 'giant' because it can be orders of magnitude greater than that of conventional magnetic alloys. The extreme sensitivity of the electrical resistance to a magnetic field make these heterostructures suitable as magnetic-field sensors, such as are needed to read the magnetic bits in hard-drive media. For the future, this same principle is being adapted to store information in addressable magnetic memory arrays known as MRAM (magnetic random access memory). A key advantage over semiconductor-based memory (conventional RAM) would be the elimination of the time-consuming boot-up when the computer power is initially turned on because, unlike RAM, MRAM is non-volatile memory.

This brings us to the task of unfolding the physics that governs such magnetic structures and phenomena. The Kawakami *et al.*¹ experiment involves modern sample fabrication techniques and a 'third-generation' synchrotron radiation source. The authors use spatially resolved photoemission spectroscopy to help them see the spin-polarized quantum-well states within the copper spacer (Fig. 1). By studying samples of variable thickness, they confirm that the quantum-well states are modulated by an envelope function (of longer wavelength).

To appreciate this work, consider that in metallic magnets the electronic spin-up and spin-down levels (or bands) are separated in energy. In non-magnetic metals such as copper the situation is different and the corresponding spin-up and spin-down bands are degenerate in energy (as a consequence of

Quantum engineering

Probing magnetism in the well

S. D. Bader

On page 132 of this issue, Kawakami *et al.*¹ report photoemission images of quantum-well states in copper thin films. Quantum-well states arise whenever electrons are confined in a small space, such as within ultra-thin semiconductor layers; the effect is analogous to quantizing electron energies in the atom. The interest in producing quantum-well states in metallic thin films can be justified in two words: magnetic recording. The past decade has seen a well-spring of new materials that exhibit unusual properties relating to the needs of the magnetic-recording community.

Information storage has to keep pace with computer development, or a bottleneck will develop that may threaten progress. So, the needs of the marketplace have fostered intense research and development in materials used not only for semiconductor processors but also for magnetic storage. Fortunately, an intellectual synergy has emerged as well. This synergy has been led by the semiconductor community's drive to exploit concepts of quantum confinement to create new devices. In nanometre-scale systems, elec-

trons have had their movements restricted in two dimensions (quantum wells), in one dimension (quantum wires) and even in all three (quantum dots). In the semiconductor field this has given rise to the 'photonic' era of 'bandgap engineering': to build structures that process light signals rather than electronic signals. In the magnetic nanostructure field we foresee an analogous 'spintronic' era of 'wavefunction engineering' — making use of the electron spin as well as its charge. This can already be glimpsed in the paper by Kawakami *et al.* and in the direction taken by many metallic-magnet researchers.

It is significant that magnetic nanostructures are becoming more sophisticated now, after a decade that gave rise to the 'giant magnetoresistance' effect², when a small magnetic field produces a large change in electrical resistance. This phenomenon is starting to be used in a new generation of magnetic heads for reading hard disk drives. The idea here is to separate, on the nanoscale, two magnetic layers by a metallic spacer. The spacer is supposedly a non-magnetic material such as copper, but its presence mediates

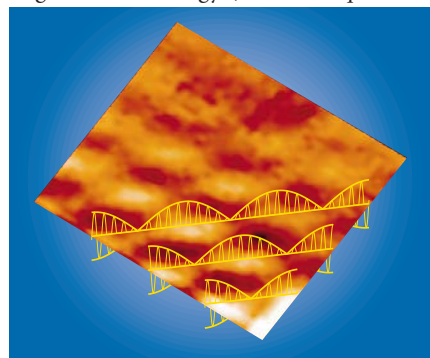


Figure 1 Imaging the quantum well. Photoemission image of magnetic quantum-well states from the work of Kawakami *et al.*¹. The dark regions identify nodes in their envelope function as shown schematically in the overlay.

the Pauli exclusion principle, one of the fundamental tenets of quantum mechanics). When the magnetic layer and copper are brought into proximity, bonding (or electronic hybridization) can occur if the energy levels match across the interface. The unique feature is that, because of the magnetic energy splitting, if one of the copper spin states can hybridize with its magnetic counterpart, then the other is mismatched in energy, and hence quantum confined. The properties of such a 'spin-polarized' quantum-well system govern the strength of the magnetic coupling³⁻⁶, which is relevant in determining device characteristics in magnetic recording applications.

These quantum wells are novel in that although they are analogous to their semiconductor counterparts, they differ in one fundamental way — they are spin-polarized, or magnetic. So, this work provides a fresh way of looking at a rather abstract concept. The data are sufficiently rich that the researchers are able to go full circle, and summarize the underlying physics in terms of an

abstract, mathematical 'envelope' function. This envelope function describes the period of the oscillatory coupling between parallel and anti-parallel spin alignment of the magnetic layers. Without going into too much detail, it is worth noting that this ingenious experiment provides a hint of what to expect from the new generation of bright X-ray sources such as the synchrotron at Berkeley. The confluence of artful sample preparation and probing techniques in this work provides a way of seeing quantum size effects in magnetic films. The basic knowledge gained can help unravel the physics of new materials for the information revolution. □

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Carbon cycling

The mysterious missing sink

David W. Schindler

I can remember discussing the implications of early data¹ on atmospheric CO₂ almost 30 years ago. Geochemists were confident that uptake of carbon at the ocean's surface could explain the imbalance between carbon released by man's activities (then considered to be almost entirely the burning of fossil fuels) and measured concentrations in the atmosphere. The rather large seasonal oscillation was regarded as a terrestrial signal, but one that remained in balance from year to year. Studies in the 1970s and 1980s, however, showed that both of these assumptions were incorrect. Detailed surface carbon-flux measurements were made at many sites in the world's oceans and, as fluxes for different regions became more precisely known, estimated average fluxes dwindled. In 1990, Tans *et al.*² announced that the annual flux to the oceans was likely to be less than 0.5×10^{15} g of carbon per year — a quarter the amount necessary to balance the global carbon budgets.

So where is the 'missing sink' for atmospheric CO₂? On page 145 of this issue, Nadelhoffer *et al.*³ provide convincing evidence that, just as it cannot be explained by the oceans, neither can the missing carbon be rationalized by uptake in the northern temperate forests — until now, the favoured theory.

The conclusion that the missing sink was terrestrial forests in the northern hemisphere⁴ came about because, after several decades of measuring atmospheric CO₂, the

amplitude of the annual fluctuation was found to be increasing. The increased flux to the atmosphere was calculated to be largely from biomass burning⁵. But the increased

release of carbon to the atmosphere was more or less balanced by increased annual uptake. Comparisons of the ¹³C/¹²C ratio in various parts of the upper ocean with carbon emitted by fossil-fuel burning reinforced the conclusion⁶.

It was assumed that either increasing atmospheric CO₂ or increasing nitrogen would drive carbon uptake in the forests. Circumstantial evidence indicated that nitrogen was involved. Not only is it the nutrient that most limits growth in the northern forests, but human releases of nitrogen to the atmosphere have more than doubled natural sources this century. Over 5×10^{12} g of nitrogen fall annually on the world's forests, and wind patterns carry this airborne fertilizer over large forested areas of eastern North America and northern Eurasia⁷. Studies in Europe documented a 25% increase in carbon uptake by forests during the period of nitrogen increase⁸. And calculations based on plausible carbon:nitrogen ratios indicated that nitrogen-stimulated 'greening' in northern forests could account for the missing sink⁹. Finally, many large-scale studies have shown that increased atmospheric CO₂ alone is unlikely to be enough to explain the increased carbon sequestration¹⁰.

Nadelhoffer *et al.*³ now show that nitrogen is not stimulating forest carbon uptake in temperate forests — the very areas where it seemed most likely. In 18 large-scale controlled experiments at nine sites in Europe and North America, the authors added ¹⁵N

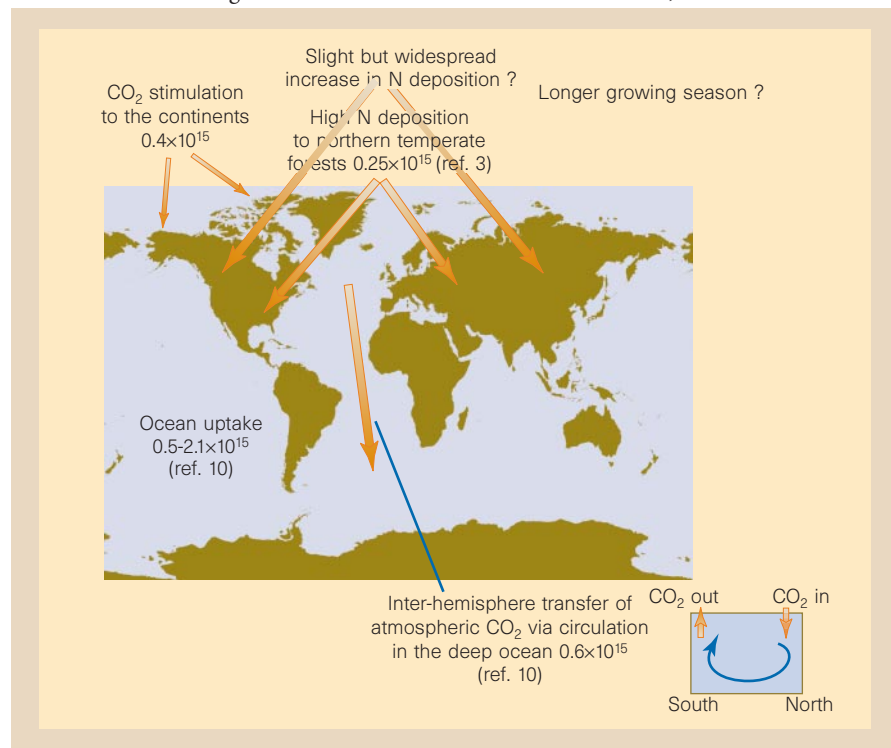


Figure 1 The global carbon whodunnit — where is the missing sink? The possibility of a single culprit has been all but dismissed by Nadelhoffer *et al.*³. Estimated annual uptake by various processes is shown, but 10^{15} g of carbon cannot account for all of the missing carbon. Question marks indicate that no estimates have been made.

and traced it through various forest compartments. Some catchments were exposed to regional nitrogen deposition; others were fertilized with nitrogen. In still other areas, nitrogen input was reduced by catching precipitation falling through the forest canopy on transparent roofs and removing most of the nitrogen. An average of only 20% of the added ^{15}N was retained by plant tissues. Just 5% entered woody tissues with a high carbon:nitrogen ratio, most going to relatively low carbon:nitrogen soils. Nadelhoffer *et al.* estimate the maximum contribution to the missing sink for atmospheric CO_2 to be a disappointing 0.25×10^{15} g of carbon per year.

So where in the biosphere is the missing carbon hiding (Fig. 1)? There are several possibilities. First, perhaps simple surface fluxes of CO_2 underestimate the role of the oceans. Broecker and Peng¹⁰ suggest that CO_2 is transported, via the deep ocean, from the North Atlantic to the southern oceans, where it is vented to the atmosphere. Second, it is possible that northern temperate forests that were stimulated by nitrogen in the past are now saturated with this element. Deposition of nitrogen in these regions has increased several-fold in the past few decades⁷. But if this picture is correct, it does not bode well for future carbon uptake in the terrestrial biosphere. Third, maybe other northern ecosystems are important in carbon uptake. Boreal forests approach the size of tropical forests — both in area and as a carbon reservoir — if peat deposits are considered. Large tracts of northern wetlands are probably very nitrogen-limited, if the few experimental studies are typical¹¹, although not much of the nitrogen released to the atmosphere currently falls in such areas.

Finally, perhaps there has been a small but widespread increase in forest growth stimulated by continuous warming in northern regions. Growing seasons in many boreal areas have increased by 2–3 weeks over the past few decades, and warming in central and western boreal regions of North America has ranged from 1–2 degrees since the early 1970s. But the most likely possibility is that the missing sink will turn out to be several small sinks — perhaps a combination of some of the above. Unfortunately, modern methods are still inadequate to identify global sinks of less than 0.5×10^{15} g carbon per year with confidence.

To a patient scientist, the unfolding greenhouse mystery is far more exciting than the plot of the best mystery novel. But it is slow reading, with new clues sometimes not appearing for several years. Impatience increases when one realizes that it is not the fate of some fictional character, but of our planet and species, which hangs in the balance as the great carbon mystery unfolds at a seemingly glacial pace. □

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Materials science

Asymmetry the easy way

Samuel P. Gido

From the atomic scale to the macroscopic, symmetry pervades nature. Crystalline materials, for example, can be described by any one of 230 space filling symmetry groups, which optimize energetic interactions between atoms or molecules. Self-assembled systems generally form with a point, line or plane of symmetry. This natural tendency towards symmetric structures has been the bane of self-assembled systems in applications ranging from liquid-crystal displays to frequency modulators to optical switches. On page 137 of this issue, Stadler and colleagues¹ demonstrate a novel approach to producing a material that self-assembles to form a non-centrosymmetric layered nanostructure.

Materials with bulk electric polarization can display many properties, such as non-linear optical effects and switchability. Whereas individual molecules can have a permanent dipole or magnetic moment, in a large assembly of such dipoles, the orientations of consecutive molecules oppose one another, cancelling out any permanent moment in the material. External magnetic or electric fields can be used to overcome the energetics underpinning the alternating or random orientation of dipoles in a material, but slow, inexorable relaxations tend to drive the system to its lowest energy state — that is, with no permanent net dipole. In addition, such poling processes increase fabrication complexity and thus cost, reducing the likelihood of practical application.

Much effort has gone into using surfaces or interfaces to force a local asymmetry due to the abrupt termination of a material^{2–4}. However, these effects are too small to produce orientation of a bulk material, and remain, for the most part, of academic interest. Alternatively, extensive synthetic procedures have been used to prepare rod-coil polymers, which form macroscopic arrays of nanostructures, arranged in a non-centrosymmetric manner⁵. The advantage of the approach taken by Stadler and co-workers² is that subtle macromolecular interactions are tailored to produce a self-assembling asymmetric structure from a blend of two simple and potentially inexpensive block copolymer materials. Block copoly-

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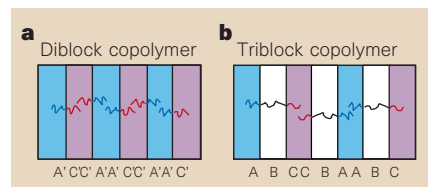


Figure 1 Diblock and triblock copolymer morphologies. Chemically different polymers are generally incompatible, so low-energy AA and CC contacts are preferred to AC interfaces. This results in the formation of centrosymmetric structures for both the diblock, a, and triblock, b. The orientations of the polymer chains associated with adjacent parallel interfaces are rotated by 180° with respect to one another in order to maximize the association of similar block materials.

mers are produced by covalently linking together two or more chemically distinct polymer blocks, each composed of a single type of monomer unit. By carefully selecting different types of materials, block copolymers can be tailored for applications ranging from the exotic to such everyday materials as removable adhesive pads and the soles of running shoes.

Imagine a polarized system that spontaneously orders into a non-centrosymmetric structure after simply being heated to a temperature where the molecules can flow. One strategy for doing this, which has achieved some success, is to marry an electrically anisotropic dipole to a molecular structure that wants to align itself owing to its shape (liquid crystallinity) or amphiphilic character (block copolymers)^{6–9}. The diblock or triblock copolymers in Fig. 1 are themselves chemical dipoles. These chemical dipoles point from one block material to the other along the trajectories of the polymer chains. The energetic (enthalpic) driving force that associates A blocks with A blocks and B blocks with B blocks leads these materials to self-assemble into morphologies in which the net chemical dipole cancels out. If one could persuade the chemical dipoles of a block copolymer system to self-assemble into an asymmetric structure they could potentially carry an optically active group along with them, producing an optically

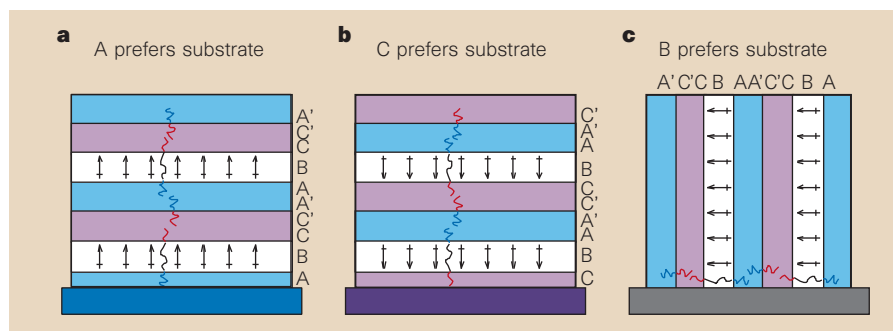


Figure 2 Diblock/triblock copolymer blends. Stadler and colleagues¹ have made non-centrosymmetric copolymer structures by combining ABC triblock and A'C' diblock copolymers. a, If the A block has the lowest interfacial energy with the substrate then the system will self-assemble into a multilayered structure parallel to the surface with a permanent dipole pointing up. b, If C has the lowest interfacial energy, the dipoles point down. c, If the B block has the lowest interfacial energy, the structure will assemble perpendicular to the substrate, with the dipoles oriented parallel to the surface.

anisotropic material useful for device applications.

The work of Stadler and colleagues¹ shows how simple but ingenious polymer physics can produce a non-centrosymmetric nanostructure from a blend of triblock (ABC) and diblock (A'C') copolymers, composed of readily accessible polymer components (Fig. 2). Here A' and A denote blocks of the same polymer material as part of the diblock and triblock, respectively. The concept is sufficiently general that one can picture its extension to a range of systems — from the optical, where polymers with large anisotropies along the chain axis could form macroscopically oriented arrays, to the biological, where anisotropic membrane structure could be tailored to promote unidirectional transport.

By themselves, the ABC triblock and A'C' diblock copolymers both produce centrosymmetric layered structures that repeat as (ABCCBA)_n and (A'C'C'A)_n, respectively (Fig. 1). The symmetry of these structures is driven by the need to localize the non-chemically bonded interactions between molecules at interfaces buried in the centre of the A and C material domains; the block copolymer chemical dipoles need to oppose one another. The combination of these diblock and triblock copolymers leads to the formation of a non-centrosymmetric structure (Fig. 2) with a repeating sequence of the domains (ABCC'A)_n. The ABC and A'C' molecules can be imagined as chemical dipoles of different strengths, which oppose one another in the non-centrosymmetric sequence, but overall produce a finite moment. Of course, this only occurs when there are additional interactions in the system that favour AA' and CC' contacts over AA and CC contacts. In this case, a preference for asymmetry in the interactions between polymer chains tethered to opposing interfaces breaks the symmetry of the structure.

Thin-film applications of these asymmetric self-assembling systems should be pos-

sible. The interfacial interactions between the individual block materials in the diblock or triblock copolymer at a solid interface or at a free surface will dictate the specific orientation of the multilayered structure¹⁰ and, therefore, the asymmetry of the properties. If we assume, for example, that the B block of the triblock carries a net dipole pointing from

its interface with A to its interface with C, then, depending on the substrate, different orientations of the overall dipole can be produced (Fig. 2). It is apparent, whether one is dealing with dipoles, chiral monomers in the chain, or directional interactions, that the concepts developed by Stadler and colleagues point to a very direct, robust and easy means of achieving asymmetry, but letting nature do the work for you. □

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Genome structure

Retroshuffling the genomic deck

Jef D. Boeke and Oxana K. Pickeral

The shape of modern genomes reflects evolutionary forces that have operated on them for millions of years. According to the exon-shuffling hypothesis, which was first suggested in these pages over 20 years ago¹, new genes are assembled from chunks of old ones. But the molecular mechanisms by which these exons (protein-coding sequences) could be glued together remained speculative. A report in *Science* by Moran and colleagues² now suggests an unexpected and efficient source of such genomic reshufflings. The authors looked at the abundant L1 (LINE-1) retrotransposons — transposable elements that can replicate within the mammalian genome using reverse transcriptase, which copies the retrotransposon RNA into DNA³. L1 usually moves only its own sequence from one genomic location to another (Fig. 1a). But Moran *et al.* show that it can, with surprising efficiency, co-mobilize a 3' flanking segment of non-L1 DNA to new genomic locations. In this way, two previously unlinked host genomic segments are juxtaposed in a process referred to as 3' transduction (Fig. 1b).

The process of 3' transduction may occur in many eukaryotes, and several possible examples of it have been reported. In the most convincing case, a progenitor human L1 element, which spawned an insertion mutation in the *dystrophin* gene, was identi-

fied based on its unique, transduced 3' flanking sequence tag⁴. A more ancient event, involving a human *CFTR* exon, probably occurred around ten million years ago⁵. In mouse cells, an L1 read-through RNA has

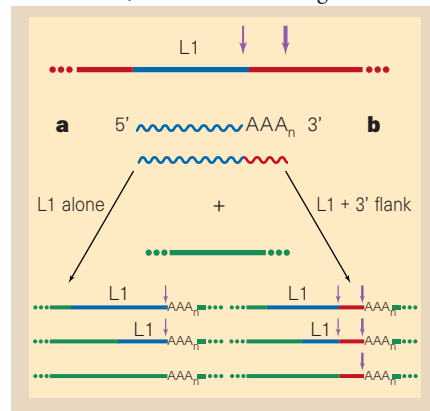


Figure 1 Mechanism of 3' transduction by the L1 retrotransposon, based on the results of Moran *et al.*². Read-through transcription by RNA polymerase leads to movement of the DNA that flanks L1. The signal for terminating transcription through L1 is either the weak cleavage/polyadenylation signal of L1 itself (a), or a stronger polyadenylation signal in the 3' flanking DNA (b). When the resulting complementary DNA integrates into a new genomic locus (green), a simple L1 insertion (a), or a chimaeric insertion (b) is formed. Purple arrows, polyadenylation signals.

been found associated with ribonucleoprotein particles⁶. And the structure of a *Drosophila* gene, *jingwei*, was proposed to arise from insertion of the complementary DNA for an alcohol dehydrogenase gene into an unrelated gene⁷. There are no retrotransposon sequences in *jingwei*, but its structure is consistent with a 3' transduction event in which reverse transcription terminated before retrotransposon sequences were reached (Fig. 1a).

Retroviruses can incorporate cellular genes into their genomes, probably by aberrant read-through transcription into adjacent host DNA. But L1 elements differ from retroviruses in that they do not form the precise 3' ends that are normally specified by retroviral long-terminal repeats. The signal for 3' end formation comes instead from cleavage/polyadenylation signal sequences. Although the human L1 element contains a putative polyadenylation signal near its 3'

end, its sequence is not optimal — if it functions at all. This weak polyadenylation signal is supposedly suppressed by the action of more potent polyadenylation signals in the flanking DNA, resulting in the formation of a read-through transcript which could then act as the template for reverse transcription^{4,8}. By this hypothesis, the nature of the flanking DNA dictates which 3' end gets formed. This built-in sloppiness has rendered L1 an efficient exon-mobilizing machine.

Although the exact molecular mechanism of L1 retrotransposition is not known, L1 probably cuts the target DNA and then reverse transcribes its own RNA to DNA^{8–10}. To study L1, Moran *et al.*² placed a reporter gene containing a splice acceptor—but lacking 5' exon and promoter sequences—into an intact L1 element. They then introduced this contraption into cultured cells. The modified L1 very efficiently produced derivatives that had

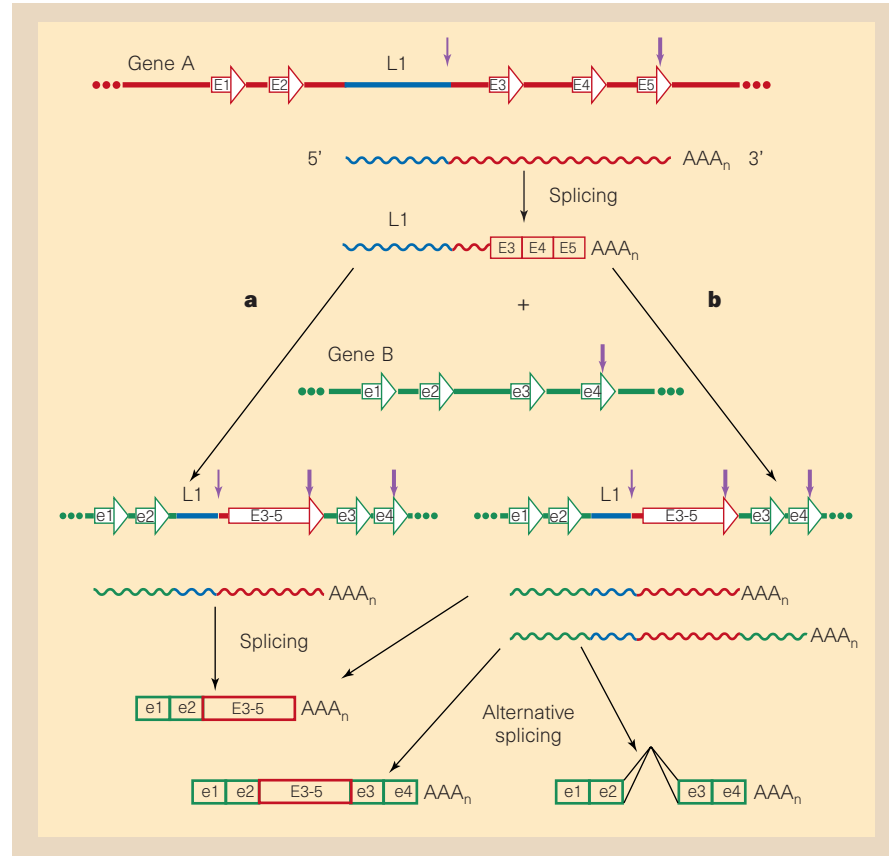


Figure 2 Evolutionary consequences of L1-mediated 3' transduction. If exons (open arrows) of gene A are downstream of an active L1 element, they can be incorporated into a chimaeric (L1–A) pre-mRNA produced by L1 read-through transcription. This transcript terminates when it encounters gene A's strong polyadenylation signal. Processing of the pre-mRNA yields an mRNA consisting of L1 fused to the distal A exons. When the resulting L1–A cDNA is inserted into an intron of gene B, a long 3' A exon including the strong polyadenylation signal is added to B. Even if inserted into the middle of gene B, this long A exon may become the terminal exon of a new hybrid B–A gene (this could help explain why 3' terminal exons are often much longer than internal exons¹¹). The distal exons of gene B will be lost if cleavage/polyadenylation at the newly introduced gene A signal is 100% efficient (a). But inefficient cleavage/polyadenylation might produce a longer primary transcript that could be alternatively spliced (b). In this way, additional mRNAs can be formed through alternative splicing of the longer primary transcript, and the organism can sample a new gene variant while maintaining the original design. This provides a new hypothesis for the genesis of alternative splicing. (Vertical arrows point to the location of polyadenylation signals.)



100 YEARS AGO

From a geographical point of view, the fundamental problem attached to the South Polar region – the verification or disproof of the existence of such a continent – is still unsolved. No less important questions likewise await solution with respect to the geological structure and character of the southern lands – so important in connection with a knowledge of volcanic action and the supposed former connection of South America with Australia – and with respect to the conditions of inland ice. ... The present seems a particularly favourable period for the resumption of South Polar research, by reason of the unusual amount of drift-ice which has within the last few years broken away from the main mass. This, together with the fact that we are now, according to Supan, passing through a warmer temperature-period, should make the next few years unusually favourable to navigation, and suggests as the most suitable starting-point for an expedition the Southern Indian Ocean, where drift ice has been particularly abundant since 1894. ... In the spring an advance would be attempted southwards over the ice and towards the Magnetic Pole.
From *Nature* 9 March 1899.

50 YEARS AGO

At the annual meeting of the Board of Greenkeeping Research ... the director of the St. Ives Research Station, Mr. R. B. Dawson, presented his report on the Station's activities during 1948. In spite of a shortage of staff, the work has increased, and research has continued on many of the old experiments put down early in the Station's history. New work has been done on soil stabilization with bitumen, seeds mixtures, growth substances for the control of weeds, and worm control. ... In addition to the pH, lime requirement and the phosphate status, the reports of the laboratory analyses of club samples now include the potash status. Golf clubs in Great Britain have continued their support, and there has been a marked increase in the support from municipalities and clubs. Educational work has been kept up in that many lectures have been given to outside bodies, and six courses of instruction for groundsmen have been arranged at the station.
From *Nature* 12 March 1949.

mobilized the reporter construct into new genomic locations. As demanded by selection for reporter-gene expression, these new sites were transcriptionally active, and each had 'trapped' the reporter-gene exon as part of a different chimaeric messenger RNA expressed in these cells.

As well as showing that L1 can transduce 3' flanking sequences into target genes, the new work illuminates aspects of L1 retrotransposition. For example, the authors estimate the frequency of L1 retrotransposition into actively transcribed genes to be at least 6%. The 3' transduction process also provides a convenient explanation for peculiarities of L1 structure, such as the dramatic variability in 3' untranslated sequences of different L1 subfamilies — they probably arise from different 3' transduction events, followed by internal deletions. Similarly, it is plausible that 5' transduction events could occur, explaining the great variability observed among 5' end sequences in different L1 subtypes. If a cellular promoter read into a full-length or partially truncated L1 element, the result would be a new 5' end for the element. Finally, 3' transduction explains the puzzling lack of target-site duplications for many L1 elements.

Moran and colleagues' results² indicate that read-through transcription of L1s is surprisingly efficient, even when the reporter gene's preferred polyadenylation signal is as much as two kilobases from the native L1 3' end. This L1 read-through transcription — terminated by a strong polyadenylation signal in 3' flanking DNA, subsequent processing, reverse transcription and incorporation into a new locus — provides a new mechanism for exon shuffling. Furthermore, this type of exon-shuffling mechanism allows fresh insights into the evolution of splicing, allowing us to tackle questions such as how alternative splicing arose, and why terminal exons are often unusually large¹¹ (Fig. 2). Until now, DNA recombination between introns was considered the best mechanism for exon shuffling. But this new mechanism implicates retrotransposons as being pivotal in large-scale modifications of gene structure.

Because most of the mammalian genome is made up of DNA that does not encode proteins, the consequences of L1 transporting such 'non-exonic' DNA are also notable. Although rearranging exons creates new proteins, L1-mediated shuffling of regulatory sequences may be equally crucial in evolution of the mammalian genome, concocting increasingly complex regulatory circuits. Changes in the timing and cell specificity of gene expression may facilitate fast morphological innovations, some of which could be enough to produce new variants or even species. The process of 3' transduction is the latest in the series of fanciful twists of logic that typify the retroelements — they have

found yet another way to mould not only their own fate, but also that of their host genome. □

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Holocene climate

Restless carbon pools

Philippe Ciais

The global carbon cycle is a significant object of study, because carbon dioxide (CO₂) is the most important greenhouse gas in the atmosphere, after water vapour. The CO₂ concentration in the atmosphere has risen by 80 parts per million by volume (ppmv) over the past two centuries¹, increasing the Earth's radiative forcing by 1.6 Watts m⁻², which causes a net reduction in outgoing infrared radiation, and it is expected to double from current levels during the next century. The concentration of atmospheric CO₂ increased by rough-

ly the same amount (80 ppmv) between the Last Glacial Maximum, 18,000 years ago, and the Early Holocene, 11,000 years ago². In-between, for the period of the Holocene spanning from 11,000 years ago to 1,000 years ago, reliable CO₂ data have not been available. This gap is now bridged in the article by Indermühle et al.³ on page 121 of this issue.

Previous measurements of air bubbles enclosed in polar ice sheets show that the atmospheric burden of CO₂ increased by 170 GtC (1GtC = 10¹⁵ grams of carbon),

Animal behaviour

Mothers of invention

What extra quality do animals that work out how to exploit new food sources have? If you're a guppy, it seems to help if you're small, hungry and female (*Anim. Behav.* 57, 331–340; 1999).

Kevin Laland and Simon Reader set up lab populations of the guppy *Poecilia reticulata* (below) consisting of males and females and hungry and well-fed fish. The fish were then put in a maze with food at the end of it. In three-quarters of the trials, a female was the first to discover the novel food source. Two-thirds of the time, it was a hungry fish that solved the problem. In a separate experiment the authors found that smaller fish were faster problem-solvers. There is some evidence that new behaviours can spread by imitation.



These results show that, rather than innovators being inherently creative, a fish's circumstances can drive it to experiment. Hungry fish are more active, better motivated to find food, and more likely to take risks than their better-fed counterparts. Small fish might do better to discover new food than fight with bigger fish for existing food sources.

Why, then, should females be smarter? Laland and Reader speculate that this is a consequence of motherhood. Guppies give birth to live young, so females, which are almost permanently pregnant, need extra food to nurture their brood. Males are probably better employed seeking out new mates than new food sources, so we might expect to see more innovative behaviour in this area of activity — something that could apply across the animal kingdom, as it is common for females to invest more in their offspring than males.

But it seems that innovators are born, as well as made. Fish that were 'past innovators' cracked new problems significantly quicker than 'past non-innovators'. So — starving artists take note — although perspiration helps, it's best to have a spark of inspiration too.

John Whitfield

between the Last Glacial Maximum and the Early Holocene². This is equivalent to a growth rate of 0.02 GtC per year during the period of deglaciation. In the more recent past, changes in atmospheric CO₂ have been observed during the last millennium, but at a rate which did not exceed 0.1 GtC per year⁴. To provide a continuous record of CO₂ over the entire Holocene, Indermühle and co-workers³ carefully analysed more than 400 precious pieces of ice from the Taylor Dome ice core in West Antarctica. The level of experimental precision in their measurements, on the order of 3 ppmv, is unprecedented.

In the new Holocene record, atmospheric CO₂ first decreased slightly during the Early Holocene, but has since increased by 25 ppmv. So, from 8,000 years ago to the eighteenth century, the atmospheric reservoir appears to have gained 50 GtC. Yet, the growth rate of CO₂ at that time is less than 0.01 GtC per year. In comparison, we now experience a build-up of CO₂ in the atmosphere at an average rate of 3 GtC per year, which clearly reflects an imbalance between sources and sinks that has absolutely no equivalent in the recent geological past.

The Holocene CO₂ record is fascinating because it reveals that small, but persistent, changes in the atmospheric CO₂ concentration can be sustained through several millennia, despite the fact that, overall, the global climate conditions have been rather stable. What are the carbon pools that have leaked carbon into the atmosphere since around 7,000 years ago? And what are the mechanisms that delivered CO₂ to the atmosphere?

Two principal active carbon pools, the oceans and the land biosphere, are connected to the atmosphere. To identify the respective role of these two reservoirs on the observed Holocene CO₂ variations, Indermühle *et al.*³ rely on measurements of the stable ¹³C isotope composition ($\delta^{13}\text{C}$) in atmospheric CO₂. The bottom line is that the $\delta^{13}\text{C}$ of atmospheric CO₂ provides a fingerprint of the terrestrial CO₂ fluxes. Photosynthesis incorporates preferentially the lighter ¹²C isotope into plant tissues. As a result, living biomass and soil organic matter are significantly depleted in ¹³C compared with the atmosphere. Therefore, if the land biosphere leaks CO₂ by respiration, it is expected to cause the global atmospheric $\delta^{13}\text{C}$ to decrease. In contrast, an ocean outgassing of CO₂ will not significantly alter the atmospheric isotopic composition.

Measuring $\delta^{13}\text{C}$ in addition to CO₂ concentration makes it possible, in principle, to infer separately the net ocean and land CO₂ fluxes. This technique is known as 'double deconvolution'⁵, but the use of ¹³C is not quite straightforward. First, it is difficult to accurately measure $\delta^{13}\text{C}$ in air enclosed in ice, and slight offsets in the calibration procedures⁶ may have a large effect on the results

of a double deconvolution. Another source of uncertainty is the fact that the air-sea gas exchange of CO₂ slightly modifies the isotopic composition of ¹³C as a function of temperature. In addition, the photosynthetic pathway common in tropical grasses (C4) fractionates much less ¹³C than the 'dominant' pathway found in trees (C3). Large-scale shifts between the C4 and C3 pathways in terrestrial ecosystems could therefore have independently caused $\delta^{13}\text{C}$ to vary during the Holocene.

Indermühle and co-workers² carefully address the above points and conclude that changes in the land biospheric carbon pool are predominantly responsible — although other processes must contribute — for the observed changes in atmospheric CO₂. The land biosphere appears to have gained carbon immediately after the deglaciation and up to 7,000 years ago, and then to have leaked about 10% of its carbon content, that is about 200 GtC. The ocean was the largest final recipient of this biospheric source, and gained 150 GtC, whereas the atmosphere retained 50 GtC. Further insights on the natural carbon cycle dynamics are expected to be gained from CO₂ and isotopic measurements in high resolution ice cores being drilled in Antarctica. A detailed CO₂ history of the last deglaciation is a high priority on research agendas.

It is worth noting that during the second half of the Holocene epoch, when the biosphere appears to have lost CO₂, the ice-core record also indicates an important change in the atmospheric concentration of methane⁷. This has been attributed to increasingly arid conditions in the tropics and peat development in the north. It appears that, even if the global average climate of the Holocene was rather stable, regional changes in the precipitation and temperature patterns over the past 11,000 years⁸ may have been sufficiently important to trigger some significant readjustments to the land carbon reservoir. Terrestrial and oceanic biogeochemical models of the carbon cycle, such as those used to predict future CO₂ levels, now face one more challenge: to reproduce the observed changes in the Holocene carbon pools. □

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Daedalus

Negative friction

Most lubricants are liquids. They keep the moving surfaces apart by hydrostatic pressure. But some, such as graphite and molybdenum disulphide, are solids. Their lamellar lattices have a very easy, low-friction slip-direction. Intriguingly, molybdenum disulphide works badly in moist air. At the contact-area of the moving surfaces, friction and compression heat the lubricant film; it reacts with water vapour to give molybdenum oxide and hydrogen sulphide. The reaction absorbs energy, which is drawn from the mechanism as added frictional loss.

Daedalus is now seeking the converse process. A lubricant which reacted under a roller to give out energy, would decrease the friction. Indeed, if the reaction were sufficiently energetic, the frictional loss would go negative. The lubricant would actually power the roller. DREADCO chemists are now developing this elegant and ultimately simple motor.

To generate propulsive power, the reaction must go with increase in volume. As the moving element rolls on a track lubricated with reagent, the rear of its contact-area would then be continuously 'jacked up' by reactive expansion, driving it along. Now metal oxidations run with vast expansion. Daedalus began to muse that a railway carriage ought to be propelled spontaneously by the thermally induced rusting of the track beneath its wheels — if rust were not such a bad lubricant. But graphite can also be expanded strongly, by the insertion of small molecules, including metal oxides, between its planes of carbon atoms. So DREADCO's 'active lubricant' will be a mixture of graphite and oxidizable metal dust. The transient heat of rolling contact will expand it strongly, thus accelerating the rolling.

Active lubrication will transform the design of small, low-powered mechanisms. They will run for ages on a modest supply of lubricant. Printers, cameras and innumerable office and domestic gadgets, will shed their motors, gear-trains and actuators, and be wonderfully quietened and simplified. More high-powered units will need special arrangements to replenish the lubricant and remove it when exhausted. Ultra-safe actively lubricated motors, free from heat and sparking, will colonize the fuel and chemical industries. An active road, pushing the cars along by reactive expansion beneath their tyres, is probably unfeasible. But an active skating rink should transform that energetic sport into a leisurely delight.

David Jones

Light-emitting suckers in an octopus

Squids and cuttlefish often have light organs on different parts of their bodies that consist of a complex arrangement of lenses, mirrors, irises, light guides, coloured filters and shutters¹. These extraordinary features are rare in their sister group, the octopods, in which light organs have been reported only in breeding females of two genera¹. We have discovered that blue-green light is emitted from the suckers of a deep-sea finned octopod, *Stauroteuthis syrtensis*. The light organs have characteristics of both simple photophores and suckers, suggesting that they could have evolved from suckers. The function of the light is unknown, but it may be related to the non-bioluminescent sucker signalling used by shallow-water octopods², or act as a lure for prey³.

The existence of bioluminescence in the suckers of finned octopods has been considered before but never verified. The blind, finned octopod *Cirrothauma murrayi* has small bodies that have been suggested to be light organs⁴ or nerve ganglia⁵, but no luminescence has been observed.

When stimulated, the suckers of *S. syrtensis* (Fig. 1a) emit moderately bright, blue-green light with a maximum wavelength of 470 nm (Fig. 1b), which can last for up to 5 min. Individual light organs either glow dimly and continuously, or blink on and off brightly every 1–2 s. These organs could be capable of luminescence, and no other part of the body emitted light. The suckers were unable to attach to surfaces, indicating that, unlike typical octopod suckers, they have no adhesive function.

The light organs of *S. syrtensis* are arranged in a single row along the length of the oral surface of each arm. The overall appearance of the light organs is similar to that of octopod suckers⁶. They are button-like structures raised above the surface of the arm but are not supported on stalks or peduncles.

Like suckers, the organs consist of an outer collar of columnar epidermal cells surrounding a cup-like depressed region called the infundibulum (Fig. 2a). At the centre of the infundibulum, a pore opens into the acetabulum, which, in *S. syrtensis*, is a narrow channel situated within the organ. The cells of the epidermal collar, infundibulum and acetabulum are covered by a cuticle (Fig. 2a,c). At the outer rim of the infundibulum, the cuticle is elaborated to form a circle of hook-shaped denticles (Fig. 2b). Each light organ is supported by a sub-acetabular ganglion (Fig. 2c) that connects with the main brachial nerve cord.

The overall morphology is typical of octopod suckers⁶, but ultrastructural examination shows that the radial, circular and longitudinal muscles of the infundibulum

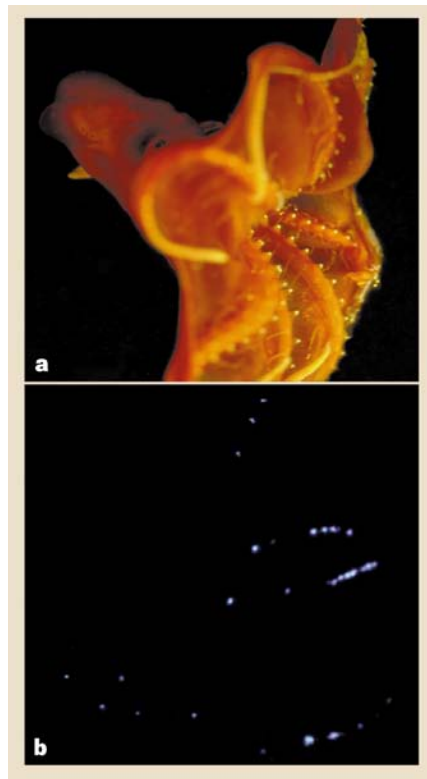


Figure 1 Views of *S. syrtensis* showing the light organs. **a**, Light organs can be seen where suckers are normally found. **b**, Digitized frame from an intensified video showing the bioluminescence. Because the video camera is monochromatic, the image has been colourized to match the actual colour of the emitted light. The views in **a** and **b** are not aligned.

and acetabulum, which are typically well-developed in suckers, are greatly reduced. The muscles are replaced by light-producing cells, or photocytes, limiting the mechanical function of the organ.

Although bioluminescence is ubiquitous in the ocean, study of its evolution has been hindered by the absence of a fossil record⁷. Light organs that retain structural traits indicative of a previous function therefore offer a rare view of their evolutionary history. The duality of sucker and photophore traits in this finned octopod supports the idea that the light organs are modified suckers. This transition of function may have occurred during colonization of the pelagic deep sea from the shallow-water benthos.

We propose that these modified suckers may have two functions: communication, and attraction of potential prey. Visual communication is almost universal in cephalopods⁸, and the suckers of some shallow-water octopods may be used in (non-bioluminescent) sexual signalling². Because the organs' wavelength of peak emission is close to that of maximum light transmission in the ocean (475 nm), they

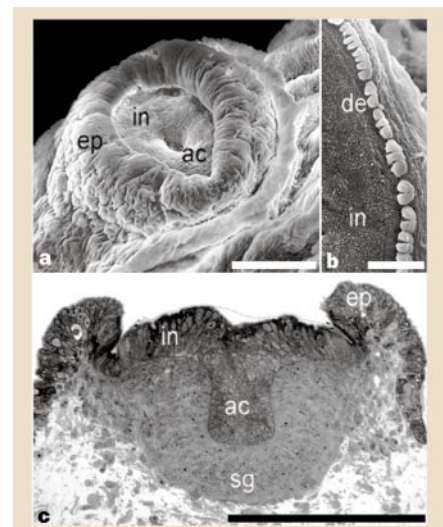


Figure 2 Micrographs of the light organs of *S. syrtensis*. **a**, Scanning electron micrograph of a whole light organ. The epidermis (ep) forms a collar around the cup-like infundibulum (in) and acetabulum (ac), which extends into the body of the organ. **b**, Scanning electron micrograph of the denticles (de), which encircle the outer rim of the infundibulum. **c**, Light micrograph of a longitudinal section through a light organ. The epidermis associated with the collar, infundibulum and acetabulum is composed of columnar cells covered by a cuticle. Presumably, light is produced by cells of the infundibulum and acetabulum. Each light organ is supported by a subacetabular ganglion (sg). The central canal of the acetabulum is not visible here. Scale bars: **a**, **c**, 100 μ m; **b**, 10 μ m.

are well suited for deep-sea communication. This matched spectrum, coupled with the common arms-spread posture of *S. syrtensis*⁹ and the location of the light organs, suggests that they may act as lures. *S. syrtensis* may trap small crustaceans in mucus produced near the mouth⁹, but as the shape of the arm web precludes any flow-through feeding current, so prey cannot be filtered from the water column, they must somehow be attracted to the mucus. Because many crustaceans are attracted to bioluminescent food sources¹⁰, the light organs may provide the attracting stimulus.

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Visualization of Bloch waves and domain walls

The theory of wave propagation in periodic structures¹ and its major concept, Bloch's theorem, have typically been assigned to the realm of solid-state physics², but they are now becoming useful in optics³, acoustics⁴ and hydrodynamics⁵. Bloch's theorem is constructive and aesthetic, starting from the idea of perfect order and dealing with infinite extended states. But Bloch's solution is unstable against a small amount of disorder.

This is particularly true of one-dimensional systems, in which Denardo *et al.*⁶ found robust localized transition regions between two extended standing-wave domains; they suggested searching for these phenomena in higher dimensions. We used liquid surface waves in which both seemingly extended Bloch states and localized domain walls can be seen alternately in two-dimensional systems.

We have taken advantage of the phenomenon that occurs when the boundary meniscus of a liquid vessel undergoing vertical vibration gives rise to a parasitic surface wave at the excitation angular frequency ω , which propagates inwards for a large distance⁷. To ensure that we worked only with such meniscus waves, the vibration amplitude of the vessel, A , is maintained below the Faraday threshold value A_c , which prevents the growth of the sub-harmonic Faraday instability^{7,8}.

We used a square methacrylate box with inner sides of 7 cm attached to the vibration exciter by an aluminium rod at one corner of the box. To modulate the liquid depth, the bottom of the box is drilled in a regular square arrangement of cylindrical holes with a radius of 1.75 mm and a depth d of 2 mm. The unit cell side is 7.5 mm. The bottom of the box is then covered with a thin liquid layer of depth h_1 (up to 0.5 mm). The liquid depth over the cylindrical holes is then $h_2 = h_1 + d$, so h_2 is between 2.0 and 2.5 mm.

To enhance the coalescence of this thin layer so that the formation of drops can be prevented, the capillary length a of the chosen liquid⁸ must be very low, 0.93 mm. The kinematic viscosity of the liquid, ν , is also very low, $0.8 \times 10^{-2} \text{ cm}^2 \text{ s}^{-1}$, so the dispersion relation for inviscid liquids^{7,8} can be

applied on an unperturbed homogeneous bottom: $\omega^2 = gk(1 + a^2k^2)\tanh(kh)$, where k is the wavenumber, g is the acceleration due to gravity, and h is the uniform liquid depth.

Although each wall generates a meniscus wave, when the vibrating rod excites from one corner, the predominant wave goes along the [110] direction. The spatial wave equation for an unperturbed homogeneous isotropic continuum is $\nabla^2\psi + (\omega^2/V^2)\psi = 0$, where $V = \omega/k$ is the phase velocity. A solution of this equation is the plane wave $\psi = C \exp(i\mathbf{k} \cdot \mathbf{r})$, where C is a constant, $\mathbf{k}$ is the wavevector and $\mathbf{r}$ is the position vector on the liquid surface.

We have used a periodic field with two slightly different velocities: V_1 on the host background and V_2 on the cylindrical holes, where $V_1 < V_2$. According to Bloch's theorem^{1,2}, the solution of the perturbed wave equation is $\psi = u(\mathbf{r})\exp(i\mathbf{k} \cdot \mathbf{r})$, where $u(\mathbf{r})$ is a modulation function exhibiting the same periodicity as the underlying Bravais lattice. We show several snapshots of seemingly

extended states aesthetically modulating the free liquid surface with the symmetry of the underlying square lattice (Fig. 1). The vibration amplitude is forced just to the limit below the appearance threshold of the Faraday instability. Such oscillatory motions could have their origin in Bloch's theorem, so that both the propagative plane wave, $\exp(i\mathbf{k} \cdot \mathbf{r})$, and the underlying modulation function, $u(\mathbf{r})$, can be clearly distinguished (Fig. 1, top). The stability of such extended Bloch states against the inevitable infinitesimal disorder of any realistic experiment might be due to the nonlinearity of our parametrically driven two-dimensional system; this is able to restore the lightly broken translational invariance, which in turn prevents Anderson localization⁶.

Then we intentionally introduce some disorder: ~4% in dimple locations and 10% in h_2/h_1 depth ratios. In this case, highly

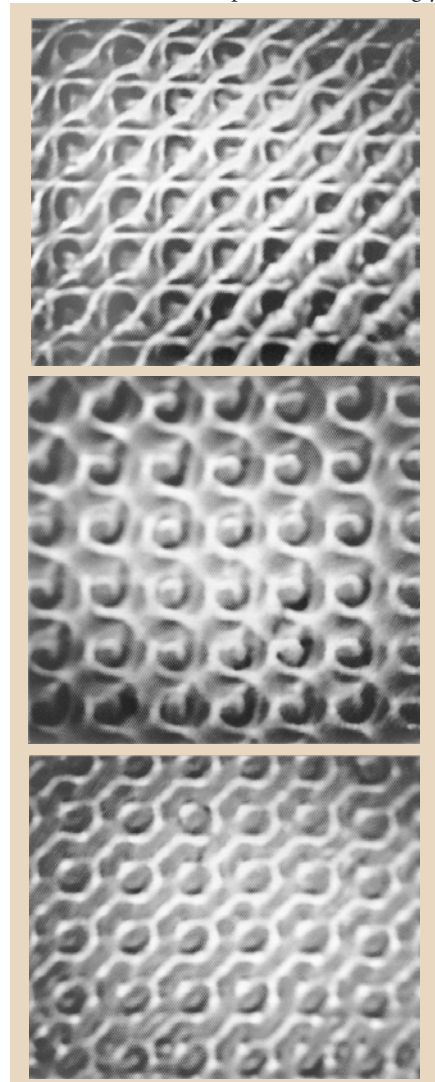


Figure 1 Extended Bloch states at excitation frequencies of 22, 30 and 35 Hz (from top to bottom). The propagative plane wave is running along the [−1,1,0] direction.

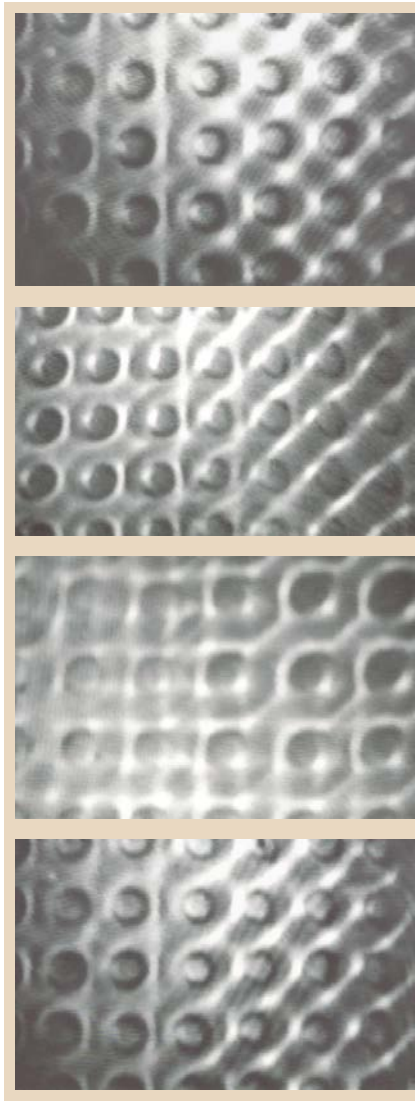


Figure 2 Localized domain walls between robust standing-wave domains. Wavevectors run along the [1,0,0] and [−1,1,0] directions with wavelengths λ and $2^{1/2}\lambda$, respectively. A sequence of snapshots is shown from top to bottom. The [1,0,0] domain is always at the left. The excitation frequency is ~20 Hz.

localized domain walls separating standing-wave regions with different but well-defined wavevectors can also be observed (Fig. 2). Thus, localization phenomena found earlier in one-dimensional vibratory systems⁶ can be generalized to higher dimensions.

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Genetic flexibility of plant chloroplasts

The chloroplast genome is thought to be monomorphic, or genetically uniform within individual plants¹. But a single plant cell may contain several hundred chloroplasts, each containing up to 900 copies of DNA², so there is a huge potential for accumulating and maintaining mutations. I found that the chloroplast genome of common groundsel, *Senecio vulgaris*, is polymorphic for a point mutation that confers resistance to triazine herbicides. Moreover, this polymorphism can vary within and among different leaves of a single plant.

Common groundsel is an annual weed found almost all over the world. It is strongly self-fertilizing, and was the first species to develop resistance to triazine herbicides³. To assess the level of chloroplast DNA polymorphism within individual plants, I used six different portions of each of five leaves of seven plants of various origin to analyse the frequency and distribution of a point mutation in a chloroplast gene that confers resistance to triazine herbicides (Fig. 1). I used the polymerase chain reaction to amplify a 277-base-pair-long chloroplast DNA fragment of the *psbA* gene spanning the point mutation conferring triazine resistance⁴.

Restriction analysis of the amplified sequence using *MaeI* resulted in two fragments of 123 and 154 base pairs in triazine-resistant individuals, and in three fragments of 35, 88 and 154 base pairs in susceptible individuals. The 123-base-pair restriction fragment is therefore diagnostic for triazine resistance and the 88-base-pair fragment indicates susceptibility; polymorphism of

chloroplast DNA is observed if fragments of both 88 and 123 base pairs occur together within a leaf sample. Sequencing of the amplification product confirmed the restriction pattern (GeneBank accession number, AF061287; data not shown).

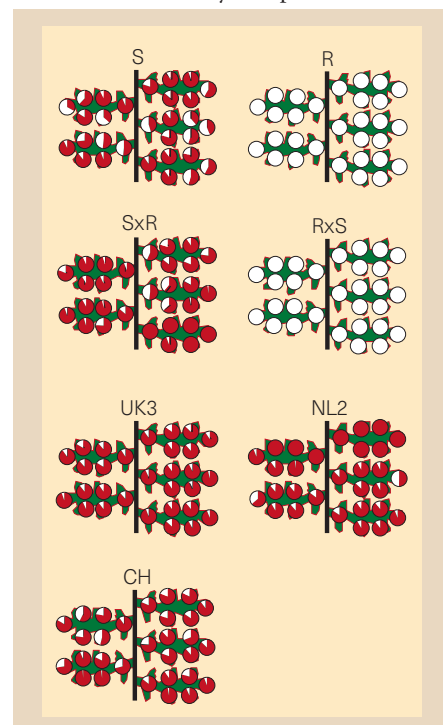
Abundant polymorphism was evident in all but the phenotypically resistant plants (Fig. 1). The pattern of polymorphism in the crosses ($S \times R$, $R \times S$) indicates maternal inheritance. Paternal leakage seems to be infrequent, as the $R \times S$ plants were sixth-generation backcrosses, indicating that there is stable transmission of polymorphic states to the progeny. The level of polymorphism in *S. vulgaris* is variable between plants ($P=0.0001$ among all plants, as well as among the polymorphic plants only; nested analysis of variance on arcsin square-root-transformed proportions⁵). Variation between different leaves is observed in some plants (NL2, $P=0.0001$; $S \times R$, $P=0.0005$; UK3, $P=0.0164$) but not others (the monomorphic R and $R \times S$; S, $P=0.2991$; CH, $P=0.0705$). Large values for residual mean squares in polymorphic plants show that considerable variation also occurs within single leaves (data not shown; see Fig. 1). Many samples showed variable amounts of additional fragments, such as a 186-base-pair fragment typically found in triazine-susceptible genotypes of other weeds⁴, indicating several other polymorphisms. Because the samples originated from different countries, chloroplast DNA

polymorphism seems to be a widespread characteristic of this plant.

Heteroplasmy (the existence of more than one type of chloroplast within an individual) may be attributed to somatic mutation or biparental inheritance, and is believed to sort out within one or very few generations⁶. Together with the fact that the point mutation that confers triazine resistance is associated with considerable fitness costs⁷, this should lead to a rapid loss of heteroplasmic states from plants. However, the resistant genotype is not rapidly eliminated from populations, even after periods without any triazine treatment, as can be seen from the genotype of the 'susceptible' laboratory-reared S line, which, since its collection in the field in 1973 and after several cycles of reproduction, still carries a considerable degree of polymorphism (Fig. 1). Assuming that paternal inheritance is infrequent in this strongly selfing weed, this indicates that transmission of the polymorphic chloroplast DNA state to the progeny can be maintained over many generations. The fitness costs of the point mutation that confers triazine resistance may therefore only be important if the level of polymorphism exceeds a certain carrying capacity.

In *Drosophila*, the evolution of heteroplasmic states of mitochondrial DNA types is affected by their fitness with respect to selective forces⁸. Within-plant polymorphism of chloroplast DNA has the potential for environmentally imposed selective

Figure 1 Analysis of chloroplast DNA polymorphism. Samples of *Senecio vulgaris* (ssp. *vulgaris* var. *vulgaris*) originated from Switzerland (CH), the Netherlands (NL2), Britain (UK3)¹⁰ and from four inbred lines from the western United States: R (triazine-resistant parental biotype), S (triazine susceptible parental biotype), $R \times S$ (triazine-resistant sixth-generation backcrossed biotype with R cytoplasm and S nuclear genome) and $S \times R$ (triazine-resistant sixth-generation backcrossed biotype with S cytoplasm and R nuclear genome)¹¹. Each leaf sample consisted of a small leaf disc (~2.4 mm²) punched out of six leaf positions of each of the first five leaves using the tip of a disposable Pasteur pipette, homogenized in 100 µl lysis buffer (20 mM Tris, pH 7.4, 20 mM EDTA, 2 M NaCl), heated three times for 5 min at 85 °C, vortexed and centrifuged. Then 3 µl extraction solution was used with fluorescently labelled primers to amplify part of the *psbA* gene, and 4 µl amplification product was digested with *MaeI* as described for several weeds⁴. Two to four restriction analyses were performed on each amplification product, and 2 µl digested DNA was analysed. Possible effects of star activity of the restriction enzyme were tested by digesting different concentrations of purified amplification products. Levels of chloroplast DNA polymorphism for individual leaf samples are shown as the median of the restriction analyses of the amplification products of the ratio between the quantity of the 88-base-pair fragment (red) and the quantity of the 123-base-pair fragment (white), based on fragment peak amplitudes. Because the undigested 277-base-pair fragment found in many analyses may be due to either incomplete digestion or a resistant genotype lacking the 154-base-pair restriction site, the index probably underestimates the relative frequency of resistant genotypes.



change in chloroplast gene frequency within the lifespan of an individual plant. For example, a polymorphic plant that survives herbicide treatment will probably have eliminated susceptible chloroplasts, and will be insensitive to further herbicide treatment. If this process is completed before the plant starts to develop seeds, its progeny will carry only the resistant chloroplast DNA genotype and may therefore be completely resistant to herbicides. Such a phenotypic effect has been found in *Chenopodium album*, in which sublethal treatment with atrazine of plants with intermediate resistance resulted in resistant seeds⁹. This within-plant selection between different chloroplast DNA types will occur whether polymorphism results from rare bipaternal inheritance or stable transmission.

Chloroplast DNA polymorphism therefore provides an additional level of selection that gives plants a powerful mechanism by which they can adapt rapidly to specific environments. This mechanism may in part be responsible for the very rapid evolution of triazine resistance in *S. vulgaris*.

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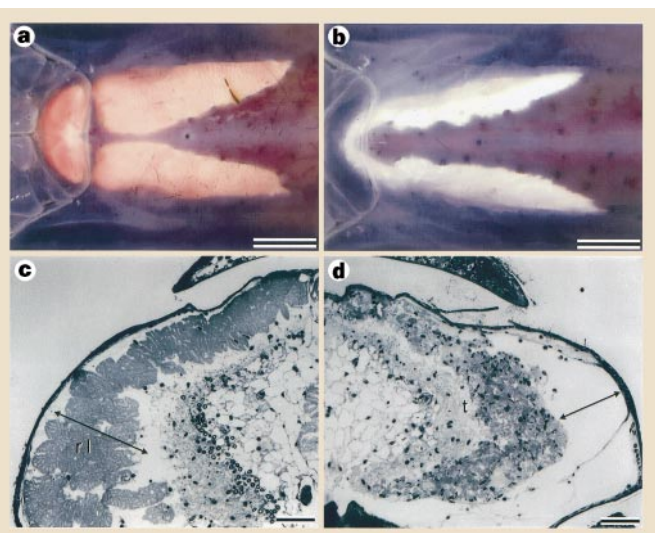
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Are vent shrimps blinded by science?

The exploration of deep-sea hydrothermal vents has depended on the use of manned submersibles, which are invariably equipped with high-intensity floodlights. But the eyes of many deep-sea crustaceans, which are exquisitely adapted for the dim conditions at such depths, can suffer permanent retinal damage as a result^{1–3}. We suggest that the use of floodlights has irretrievably damaged the eyes of many of the decapod shrimps (family Bresiliidae) that dominate the fauna at vents on the Mid-Atlantic Ridge⁴.

We collected *Rimicaris exoculata* and *Mirocaris (Chorocaris) fortunata* shrimps at the Rainbow and Lucky Strike sites, respectively, using the submersible *Nautil*

Figure 1 Eyes of deep-sea shrimp. **a, b**, The dorsal surface of live *Rimicaris exoculata* showing variations in the thoracic eye: **a**, the pink-eyed type, possibly coloured by rhodopsin; **b**, the white-eyed type, apparently with only the reflective tapetum. **c, d**, Sections through resin-embedded specimens of *Mirocaris fortunata*. **c**, In pink-eyed specimens there is an extensive rhabdom layer (rl, arrow) extending beneath the carapace from the midline to the lateral margin. **d**, In white-eyed specimens there is no rhabdom layer (arrow), although the tapetum (t) remains undamaged. Scale bars: **a, b**, 1 mm; **c, d**, 100 µm.



during the AMORES Marvel cruise of RV *Atalante* in August 1997. We captured the animals by using a suction pump in flood-light illumination and brought them to the surface in a blacked-out Perspex chamber, which provided limited protection against surface light exposure.

The thoracic eyes of some individuals were pink, with a smooth outline and regularly dappled appearance (Fig. 1a), whereas others were a matt chalky white, often with dark areas or streaks in the otherwise featureless reflector (Fig. 1b). We examined the morphology of pairs of specimens of both *R. exoculata* and *M. fortunata* with pink and white eyes, each pair taken from the same sample. The pink-eyed specimens show the normal extensive rhabdom (photoreceptor) layer^{5,6}, although there is some evidence (confirmed by electron microscopy) of recent damage to the microvilli (Fig. 1c). The white-eyed specimens of both species show severe breakdown, often with complete loss of the rhabdom layer (Fig. 1d).

The Rainbow site was discovered in 1994 by remote physicochemical sampling without illumination⁷. The submersibles *Alvin* and *Nautil* first visited the active vents in July 1997. We suggest that the retinal damage observed in white-eyed *R. exoculata*, collected just one month later, was caused by the lights used during these surveys. The shrimps swarm over the vent chimneys and are illuminated by any vehicle working at the active region. The Lucky Strike sites have been visited many times, and we believe that the differences in our Lucky Strike specimens of *M. fortunata*, most of which had white eyes, have the same cause.

The eye structures of vent shrimps have varying degrees of abnormality, usually ascribed either to poor fixation or to light damage during collection^{5,6,8}. *Alvinocaris*, for example, apparently has no rhabdoms⁹, but this may be a consequence of previous

encounters with a submersible, rather than being a specific adaptation. The only cases where damage has not been observed are those of juvenile specimens taken by trawling in midwater well above the vents¹⁰. These shrimps would not have been subject to previous floodlighting.

The rate of onset of retinal pathology is slow enough for the structure of the retina to be relatively unaffected over a period of hours (as can be seen from our illuminated pink-eyed specimens) but rapid enough for dramatic deterioration to occur in the few weeks between the initial visits to the Rainbow site and our capture of the *R. exoculata* specimens. There is at present no means of working at the vents without causing this damage, so every vent population visited will already have been exposed to it.

We have established an associative link but not a causal one. Confirmation of these conclusions will require study of the eyes of shrimp captured at the first visit to any new vent site and, ideally, an *in situ* time series of light-exposed specimens from the same site. Meanwhile, any behavioural observations at previously visited vent sites may relate to shrimp that are already blind.

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Bursting a south-sea bubble

The Fateful Hoaxing of Margaret Mead: A Historical Analysis of Her Samoan Research

by Derek Freeman

Westview: 1998. 279 pp. \$24, £16.50

Melvin Konner

The title of this book contains two claims. The first is that Margaret Mead, most acclaimed of American anthropologists, was hoaxed by her own informants during her 1928 field trip to Samoa. In support of this claim, Derek Freeman presents several kinds of evidence. Strongest is that Mead made mistakes in her ethnographic descriptions. Weakest is an interview with an octogenarian religious lady, very distressed by the loose reputation Mead had given Samoan girls. Six decades after the fact, she swore on the Bible that she had deliberately tricked Mead into thinking that she and her even younger friends were over-sexed.

There is not much to be said about this hoax claim except that it may be true. Mead was a young woman, on her first field trip, and she may have been flummoxed by some Samoan teenage girls who told her that their lives were both sexy and free of conflict. Other ethnologists in Samoa, including Lowell Holmes, Paul Shankman, Bradd Shore and Freeman himself, came along later and did better research, correcting Mead's impressions. So much was already true in 1983, when Freeman published *Margaret Mead and the Heretic: The Making and Unmaking of an Anthropological Myth* (Penguin, 1997).

Scientists make observations. They publish them, sometimes using them to support theories. Other scientists, with different methods and theories, may be sceptical. They repeat the observations. Sometimes they disprove them. They report new observations and different theories. So?

In ethnology there is an additional problem: the subjects of study are intentional agents. They are influenced by being studied — a sort of anthropological uncertainty principle. They can put up a smokescreen, conceal facts, even deliberately mislead observers. Such is the nature of all studies of humans, and it is a source of error in psychology, medicine, demography and other human sciences, as well as ethnology. As post-modernist anthropologists never tire of pointing out, it is difficult to achieve objectivity in ethnographic field studies. So?

Soon after the publication of Freeman's other book about Mead, it became clear that Samoan studies were blessed with a series of talented ethnographers who had already detected and corrected Mead's mistakes. Freeman nicely summarized their work, adding his own very useful observations of



CORBIS

Duped? But did it matter if Margaret Mead was tricked by her informants on that first field trip?

adolescent development. For example, he showed that the curve for age at first arrest for a crime among Samoan teenagers is virtually identical to that derived from similar studies in England. Thus, when Mead used Samoan teenage life to suggest that adolescent *sturm und drang* was an exclusively Western phenomenon, she was wrong.

Now Freeman has written a new book about Mead, delving more deeply into the historical material. He reviews the history of American cultural anthropology in the period before the Samoan research, and offers rich biographical detail on the young ethnographer, her relations with her famous mentor Franz Boas and other anthropologists, and especially her exuberant and sometimes naive first field trip to Samoa. He attempts to show that Mead was not just mistaken, but was deliberately tricked by her informants. His argument is well developed and to some extent convincing. Although some authorities reject it — notably George Stocking, the leading historian of anthropology, who replied sceptically when Freeman first field-

ed the claim in 1989 — let us say for the sake of the argument that we accept it.

The second claim of the book's title, that Mead's hoaxing was "fateful", is very different. It cannot be sustained by letters from the field, repeat interviews of informants in their old age, or even by follow-up fieldwork. This is not a claim about one scientist's mistake. It is a claim about the history of a discipline, and it is in no way proven in this book.

The gist of it is that Mead's *Coming of Age in Samoa* profoundly shaped anthropology in the United States, undermining truths about human nature and strengthening falsehoods about the power of culture. There is no doubt that American anthropology during the mid-twentieth century was hostile to generalizations about human nature and biological influences on behaviour. But to attribute this vast *Zeitgeist* to Mead's little 1928 book is untenable. It arose from a sound rejection of nineteenth-century racist theories (stirring again in Europe when Mead's book appeared) and a disenchantment with the view that human flexibility and choice could

in some way be cut off by biology. It was a broad intellectual thrust throughout the English-speaking world, affecting psychiatry, psychology and education as much as anthropology. Writings by John Dewey, John Watson, B. F. Skinner, Talcott Parsons, Bronislaw Malinowski, Boas (pre-Mead) and M. F. Ashley Montagu were far more influential than Mead's youthful book.

Freeman sees the controversy over that book as overwhelmingly important, saying that "Mead's demonstrably erroneous conclusions about Samoa were seriously questioned for the first time early in 1983. Indeed, before the year was out, the scientific standing of Margaret Mead's Samoan research had become the ruling *cause célèbre* of twentieth-century anthropology." Of course, this exaggerated claim gives Freeman's own 1983 book a crucial role in the modern history of the discipline.

Let me suggest some more plausible candidates for the ruling *cause célèbre* of twentieth-century anthropology: fighting racist theories, demonstrating the flexibility of sex roles, promoting respect for exotic traditions, challenging the ethnocentrism of psychologists, sociologists and historians, fighting colonialism, questioning research methods that 'objectify' non-Western people, preserving disappearing cultures and resisting the generalizations of sociobiology. To every one of these genuine *causes célèbres*, Mead made a significant contribution.

In the interests of full disclosure, I should say that Freeman takes me to task for calling Mead "one of the greatest of all social scientists" and suggesting that she might have deserved the Nobel Prize. I attributed these comments to youthful enthusiasm (they occur in a 1982 book of my own) until I checked the context. I described then-current knowledge of biological bases of human behaviour, and, in the forthcoming second edition, as in the first, my overall viewpoint on this is closer to Freeman's than to Mead's.

Yet I stand by my strong statements about her. As the context makes clear, I was moved to such praise by re-reading Mead's 1948 book *Male and Female*, which includes material not just on Samoa but on seven different traditional cultures she had studied directly. She used ethnographic data from these and other cultures to launch a frontal assault on the then-prevailing Western idea that every major aspect of gender-assigned roles stemmed from biological determinants, and was therefore inevitable and unchangeable.

Today, everyone who is not a religious fundamentalist or an unlettered boob of the male sex agrees that Mead was right and the prevailing idea was wrong. Mead's book, which preceded Simone de Beauvoir's *The Second Sex*, Betty Friedan's *The Feminine Mystique* and all the feminist sociology that followed, sowed the seeds of freedom and equal opportunity now enjoyed by millions

of women in the West and, increasingly, by scores of millions throughout the world.

Still, as my use of her work showed, she also provided the facts needed to show some of the *limits* of sex-role variability, especially in physical aggression. This does not change her fundamental conclusions, nor, certainly, the policy implications, but it modifies Mead's view to some extent. What greater tribute could an anthropologist have than to have provided ethnographic data on disappearing cultures that a later author could use to qualify her conclusion? As anthropologist Melvin Ember has said, Mead was a natural historian of human societies. A Nobel Prize went to Niko Tinbergen, Konrad Lorenz and Karl von Frisch in 1973 for work on the natural history of animal behaviour. A Nobel Prize might well have acknowledged Mead's work, which had much further-reaching consequences.

Mead published more than 30 books, of which *Coming of Age in Samoa* was the first and one of the shortest. It was very popular and it made her name, but it does not have the importance Freeman accords it in the history of American anthropology, nor even in Mead's reputation. Through her other books, hundreds of articles, museum exhibits and countless interviews and speeches, she helped make it necessary to consider the habits and practices of non-Western cultures before making generalizations and certainly before making policy.

She promoted breast-feeding when American paediatricians sought to abolish it, and opened the minds of obstetricians about natural childbirth in an era when millions of babies were born heavily sedated. She helped change thinking about child-rearing, education, sex, menopause, ageing and race, based on her own and others' fieldwork in cultures once considered too exotic to be relevant. Mead trained dozens of anthropologists and inspired hundreds of others, many of whom went on to criticize her work and challenge her views. She was opinionated, outspoken

and easy to disagree with. Provoking disagreement was part of her personal style.

She got some things wrong? Mendel recorded data too good to be true, Darwin was a Lamarckian, Freud belittled the importance of child abuse, Einstein rejected quantum theory, Heisenberg opposed "Jewish" physics and Lorenz published a scholarly article claiming that racial mixture was dangerous. Few are willing to dismiss these thinkers or diminish their contributions because of such intellectual, or even moral, lapses. No doubt it is worthwhile to point out the lapses, and it is at least of historical interest to understand how they developed. In this spirit, Freeman has made a worthwhile contribution to the history of anthropology. But Mead's reputation endures. □

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Unstable door to the future of computing

The Feynman Processor: Quantum Entanglement and the Computing Revolution

by Gerard J. Milburn

Perseus: 1998. 194 pp. \$23, £15.95

John Preskill

During the second half of this century we have witnessed staggering progress in the development of information technology. At present, the pace of progress shows no sign of slowing. But in the early twenty-first century, conventional integrated-circuit technology will approach the fundamental limitations imposed by the atomic size scale. At that stage, continued improvement in computing performance, and the continued expansion of the world economy, may hinge on the development of radically new methods for processing information.

The shape of infinity

An illustration by Robert McCall of the docking module from the Apollo-Soyuz project, taken from NASA's *The Exploration of Space* (With Works From the NASA Art Collection) by Roger D. Launius and Bertram Lurch (Stewart, Tabori & Chang, \$60).



The inescapable principles of quantum mechanics dictate that information encoded at the atomic scale ('quantum information') must differ substantially from 'classical information', such as that encoded in the characters printed on this page. In some ways, quantum effects pose serious challenges for the reliable storage and processing of information. Due to the uncertainty principle, quantum information cannot be read without being disturbed; correspondingly, it is highly unstable and cannot be copied accurately.

But the implications of quantum mechanics for the future of information technology are not all disheartening — far from it. A growing community of physicists and computer scientists are recognizing that a quantum computer (a device that processes information encoded in a quantum state) could have astonishing power. The key to that power is 'quantum entanglement': the parts of a quantum system exhibit peculiar correlations that cannot be easily simulated by any classical device. Properly exploited, quantum entanglement can unleash a new type of massively parallel processing that could never be approached with conventional silicon-based technology. Indeed, a quantum computer operating on just a few hundred atoms could perform (in effect) a number of simultaneous computations far exceeding the number of atoms in the visible Universe! Thus a quantum computer, if and when constructed, will perform in the blink of an eye certain computations that would run for eons on today's supercomputers.

A reader seeking an accessible account of these exciting developments could hardly do better than to consult *The Feynman Processor* by Gerard Milburn. (The title honours the physicist Richard Feynman, who presciently recognized the potential of quantum computing in the early 1980s.) Milburn, a professor of theoretical physics at the University of Queensland, writes in a light and inviting style (some of the jokes are actually funny), but the content of the book is serious and highly ambitious. Milburn knows his subject well, and guides the reader through the intricacies of quantum randomness, quantum correlations and quantum information processing at a surprisingly sophisticated level.

Informed opinion about the prospects for quantum computing covers a wide spectrum, as enthusiasm for the potential power of quantum information processing is tempered by awareness of the immensity of the technological challenge. Milburn sides squarely with the optimists, stating: "a quantum computer is not only possible, but inevitable. It may take decades, perhaps a century, but a commercially viable quantum computer is a certainty."

That snappily optimistic tone energizes much of the book. A fine example is Milburn's attitude towards the irreducible randomness of the quantum world. The out-

come of a quantum measurement cannot be predicted with certainty, even by someone with complete information about the system being measured. This cosmic dice game, so distressing to Einstein, is to Milburn only a cause for wonder and exhilaration; he exclaims that "the universe is an inexhaustible source of information, a bottomless reservoir of surprise".

Considering how hard Milburn works to keep the presentation entertaining and understandable, he packs an impressive amount of material into less than 200 pages, and the typical reader will be challenged to follow the carefully woven arguments in detail. The diligent reader will be amply rewarded with an assortment of insights into the elements of quantum algorithms, the perplexing properties of quantum information and the schemes for processing it that are now being pursued in laboratories around the world.

Naturally, so ambitious a book is not without flaws. For example, the discussion of quantum teleportation gives a misleading impression about the amount of classical communication required to perform this feat. I also feel that Milburn's discussion of the foundations of computation muddles the distinction between computability (in principle) and tractability (in practice). Even a careful reader might easily draw the incorrect conclusion that a classical computer is incapable of simulating a quantum computer. In fact, such a simulation is not impossible, only woefully inefficient.

Despite a few such quibbles, I recommend this gracefully written and informative book to non-specialists seeking an up-to-date, accurate and uplifting perspective on the profound potential of quantum information processing. □

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Tides in our time

Tides: A Scientific History

by David Edgar Cartwright

Cambridge University Press: 1999. 292 pp.

£45, \$69.95

Paul Melchior

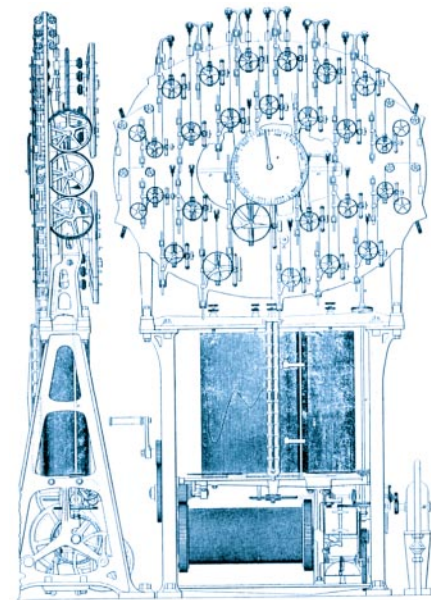
No comprehensive account of how we have come to understand the striking phenomenon of tides has been published since 1911 with the re-released edition of *The Tides and Kindred Phenomena in the Solar System* by George H. Darwin. Now comes David Cartwright's *Tides: A Scientific History*, a major contribution to the history of the astronomical and geophysical sciences, filling that serious gap in the scientific literature.

Our centuries-long struggle to identify the astronomical and geophysical causes of tides in all their diverse manifestations has not been without trials and errors. The road often led up blind alleys, but the course had become less tortuous and the path ahead clearer by the end of the seventeenth century. However, the most likely definitive solution has been found only in the past 20 years.

Cartwright reminds us that Pierre-Simon Laplace had written: "... le problème des marées est le plus épineux de toute la mécanique céleste." Just as this "thorny problem" is finding a solution, it is timely that a leading specialist should write its history. The book testifies to Cartwright's great erudition and presents considerable and often original work. His description of the observations and mathematical advances that make up the history of the science of tides is clear, rigorous and enjoyable. Over the 300 pages, none of which is superfluous, Cartwright's sequence of chapters takes on the rhythm of the history.

The author comes up with some surprises; he points out, for example, that the term 'Coriolis acceleration', which is frequently used by scientists, was introduced by Laplace before Coriolis was even born. Or, the fruit of his own research, he tells us that the first person to observe the semi-diurnal oscillation in surface barometric pressure in the tropics was Robert de Paul, chevalier de Lamanon, during the journey of the explorer J.-F. Galaup de La Pérouse in 1785.

Cartwright describes the apparently contradictory facts available in the 1800s that the Moon's motion seemed to be increasing, that the distance between the Earth and the Moon seemed to be increasing by around 3.6 centimetres every year, and



A mechanical tide predictor built in 1877–79 and designed by Edward Roberts.

that the Earth's speed of rotation was slowing down. He explains the problems of the dissipation energy budget which has intrigued astronomers since Laplace. Light was shed on all this by the study of the particular case of the Irish Sea model (1919). But a final estimate for the equilibrium energy budget had to await data obtained later by artificial satellites and lunar laser-ranging measurements.

Until the dawn of the twentieth century, tidal research was carried out almost exclusively by two European countries that maintained large navies and regularly observed the effects of huge oceanic tides on their coasts: the United Kingdom and France ("the continental writers", as Cartwright refers to them). At first astronomers played a fundamental role: in Britain several Astronomers Royal, including Harold Spencer Jones, were involved, while Laplace dominated the French scene (assisted by the astronomer Alexis Bouvard, as Cartwright subtly notes).

The development of celestial mechanics and "théories de la Lune" led to the theory of the tides and the Laplace tidal equations, which represented the first mathematical mastery of oceanic phenomena. Lord Kelvin and George Darwin extended these equations to describe the harmonic expansion of the tides. And even at that time, it was possible to use the amplitude of tidal waves to make a preliminary evaluation of the mass of the Moon.

New horizons opened with the advent of the American Rollin Harris's *Manual of Tides* in 1901 and his construction of 'cotidal lines' which covered the world's oceans. Although George Darwin was harshly critical of this work, the great mathematician Henri Poincaré gave it generous support. Again Cartwright reminds us how Harris's intuitiveness led him to propose the existence of an underwater ridge in the Arctic Ocean; this major oceanic feature was originally called the Harris ridge, but is now known as the Lomonosov ridge.

In subsequent chapters covering the twentieth century, we find oceanographers and geophysicists taking the lead from the astronomers in the field of tidal discovery. We hear about the famous Liverpool Tidal Institute, which was important, both observationally and theoretically, in providing tidal analysis and predictions so well perfected by Arthur Doodson that they delayed the use of computers in this area. In these same chapters, Cartwright approaches the difficult aspects of pure mathematics with clarity, describing, for example, the theory developed by Joseph Proudman, George Goldsbrough and Doodson to describe tides in basins and oceans as represented by simple geometrical shapes.

The last three chapters see tidal research extended over the entire world, and

describe events known to most readers, and during which Cartwright himself had a key role. This was the time when, with the introduction of computers, the first global model of ocean tides was calculated by Chaim Pekeris in 1960, when ocean tidal loading was introduced in the Laplace equations, while in 1979 the models of Ernst Schwiderski described 11-diurnal, semi-diurnal and long-period tides. During this time also, George Platzman calculated the normal modes of oscillation of the ocean, and a method for analysing and predicting tides, known as the 'response method', was developed by Cartwright and Walter Munk.

The book ends with the more recent years (one of the last references is from 1996), which see technological advances — bottom pressure recorders and superconducting gravimeters, for example, but in particular satellite altimetry, which, with the US/French TOPEX/Poseidon satellite, has attained the incredible accuracy of ± 3 centimetres in measuring the distance between the satellite antenna and the sea surface.

I really enjoyed this book, and consider it a masterpiece. I was delighted to find, among its many high-quality illustrations, George Darwin's ingenious drawing of the evolution of the Earth-Moon system, as I used to set this as an exercise for my students.

Obviously, the book is primarily written for astronomers, geophysicists and all those known as 'tidalists', who will surely enjoy it. But I also recommend it to physicists and pure mathematicians. □

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PGOMLDADLDCD

My Brain is Open: The Mathematical Journeys of Paul Erdős

by Bruce Schechter

Oxford University Press/Simon & Schuster: 1998. 224 pp. £22.50/\$25

Alexander Masters

When Paul Erdős "left" (as he called it) in 1996, even *The New York Times* took notice, and printed a front-page obituary calling him "a wayfarer in math's vanguard". Two years later, Paul Hoffman published an excellent biography about him called *The Man Who Loved Only Numbers* (Fourth Estate/Hyperion, 1998; reviewed in *Nature* 394, 535–536; 1998), which reached number three in *The Sunday Times* best-seller list. Now Bruce Schechter has followed this up with a second biography, *My Brain is Open*. In his 83 energetic years, this peculiar, obsessive little mathematician has built up a substantial public following.

Erdős was born in 1913 in Hungary, and discovered negative numbers when he was four. He belonged to that extraordinary cluster of scientific geniuses — which included John von Neumann, inventor of the electronic computer and game theory, and the Nobel prizewinners Eugene Wigner, George de Hevesy and George Olah — who emerged from two or three schools in Budapest after the First World War.

During his life, Erdős wrote more than 1,500 papers, books and articles, more than any other mathematician ever. Some of these became the great classics of our century, opening up entirely new fields of study to which generations of mathematicians have devoted their lives. As the mathematician Paul Winkler said, "If I can see a bit farther it is because I stand on the shoulders of Hungarians".

Schechter is particularly concerned to clarify a few of the basic ideas underlying this astonishing production, such as the sieve of Eratosthenes, Cantor's infinities and Euler's curious problem (the foundation of graph theory) about whether a man could walk across all seven bridges of Königsberg without crossing the same bridge twice. It is inspiring reading, because Schechter, following in the spirit of Erdős, makes clear that much good mathematics can come from whimsical speculation and the clever use of simple ideas. If popular writing about mathematics remains of this quality, there is still enough unused material for a shelf-full of Erdős biographies.

In other respects, Hoffman's book is slightly longer and better on anecdotes, but Schechter's, while not so lively, is less inclined to get distracted from its subject. Although you would never guess it from the acknowledgements, both writers once worked for *Discover* magazine. Erdős's famous love of creative cooperation has apparently not spilt over into the world of mathematical biographies.

From his teenage years, when he could instantly square a four-digit number and knew 37 proofs of Pythagoras's theorem, Erdős called himself old and decrepit. A few years before his 60th birthday he appended the letters PGOM to his name, which stood for Poor Great Old Man, then added further initials every few years. By the time he was 75 he was PGOMLDADLDCD (Poor Great Old Man, Living Dead, Archaeological Discovery, Legally Dead, Counts Dead).

His output had slowed down a little by then. "One of my greatest regrets," remarked one of his last collaborators, "is that I didn't know him when he was a million times faster than most people. When I knew him he was only hundreds of times faster." □

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Holocene carbon-cycle dynamics based on CO₂ trapped in ice at Taylor Dome, Antarctica

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A high-resolution ice-core record of atmospheric CO₂ concentration over the Holocene epoch shows that the global carbon cycle has not been in steady state during the past 11,000 years. Analysis of the CO₂ concentration and carbon stable-isotope records, using a one-dimensional carbon-cycle model, suggests that changes in terrestrial biomass and sea surface temperature were largely responsible for the observed millennial-scale changes of atmospheric CO₂ concentrations.

Precise and continuous direct measurements of the concentration of atmospheric CO₂ started in 1958, and show a clear increase from 315 parts per million by volume (p.p.m.v.) to 364 p.p.m.v. by 1997¹. This accumulation of the most important greenhouse gas after water vapour is caused by fossil-fuel burning and changes in land use. For a better understanding of the carbon cycle it is important to know the concentration history of atmospheric CO₂ on various timescales. Before 1958, such histories can only be investigated reliably by analysing air enclosed in polar ice. Earlier work showed that the atmospheric CO₂ concentration was about 280 p.p.m.v. before the beginning of industrialization² and that only small variations of about 5 p.p.m.v. occurred during the pre-industrial part of the past millennium^{3,4}. However, the atmospheric CO₂ concentration increased from about 200 to 270 p.p.m.v. during the transition from the Last Glacial Maximum (~20 kyr before present, BP) to the beginning of the Holocene (~11 kyr BP)⁵. During the last glacial period, fluctuations of ~20 p.p.m.v. occurred on a millennial timescale from 46 to 18 kyr BP (ref. 6). In contrast, little is known about the CO₂ concentrations during most of the Holocene, the current interglacial period⁷.

The stable-isotope composition of atmospheric CO₂ ($\delta^{13}\text{C}$; see Methods for definition) permits the attribution of carbon to different carbon reservoirs. The detection of the fossil-fuel signature (the Suess effect) was first reported by Keeling *et al.*⁸. Direct atmospheric measurements, firn air samples and samples extracted from ice cores revealed a decrease of $\delta^{13}\text{C}$ from -6.5‰ to -7.8‰ since the beginning of industrialization; they also showed a small century-scale variability during the pre-industrial part of the past millennium^{9,10}. Other measurements on ice cores have shown that $\delta^{13}\text{C}$ increased by ~0.3‰ from the Last Glacial Maximum to the Holocene¹¹. The $\delta^{13}\text{C}$ record, when used in inverse modelling, gives a further constraint on the global carbon budget and allows the calculation of the net fluxes of carbon from the atmosphere to the ocean and to the land biosphere^{9,12,13}.

Here we present a high-resolution record of CO₂ concentrations as measured in air bubbles trapped in an ice core from Taylor Dome, Antarctica, covering the entire Holocene. The record shows a decrease of the CO₂ concentration from 268 p.p.m.v. at 10.5 kyr BP (the end of the transition from the last glacial to the Holocene) to 260 p.p.m.v. at 8.2 kyr BP. During the following 7 kyr, the CO₂ concentration increased almost linearly to ~285 p.p.m.v. The reproducibility (that is, the standard deviation of the mean) of most of our measurements is better than 1 p.p.m.v. Furthermore, we present a Holocene $\delta^{13}\text{C}$ record from the same ice core. The record

shows an increase of 0.3‰ from 11 to 8 kyr BP, and a decrease of 0.2‰ over the following 7 kyr. On the basis of inverse modelling we propose that most of the variability in atmospheric CO₂ concentration is caused by changes in the amount of land biomass and in sea surface temperature.

Potential *in situ* and analytical artefacts

The reconstruction of atmospheric CO₂ concentration from ice cores is not a straightforward procedure owing to the following difficulties. High-resolution measurements of CO₂, and the comparison of CO₂ records from different ice cores, have shown that the atmospheric CO₂ signal can be masked by CO₂ production in the ice matrix; such *in situ* production is most likely due to chemical reactions between impurities, collectively referred to as natural artefacts. The physics and chemistry of these processes, documented in ice cores from Greenland^{14–16}, are little known, but two possible candidates are being investigated. (1) Acid-carbonate reactions^{14,16,17} and (2) oxidation of organic matter by H₂O₂ (ref. 18). The probability of a reaction depends on the concentration of the impurities and their location in the ice (for example, at grain boundaries or associated with dust particles), which are not known in detail for either Greenland or Antarctic cores. But it has been shown that the concentrations of the impurities are one order of magnitude smaller in ice cores from Antarctica than in those from Greenland, so that the probability of natural artefacts is smaller in Antarctic records^{14,18,19}. Detailed measurements on a Greenland core (GRIP) have shown an *in situ* CO₂-production potential (by an acid-carbonate reaction) of up to 3,000 p.p.m.v., but a CO₂ surplus of only 30 p.p.m.v. has been observed¹⁸. Thus, an existing CO₂-production potential does not necessarily mean that a natural artefact occurs. At present, the most promising test to detect such natural artefacts is to perform detailed measurements across a few annual layers. If the measured CO₂ concentration represents the atmospheric composition, the variation between adjacent samples should be similar to the analytical uncertainty. If CO₂ is produced by chemical reactions, a larger scatter of the data points is likely.

Fractionation of CO₂ between bubbles and clathrates may occur in the clathrate-formation zone of the ice, whose depth depends on the local temperature²⁰. If the extraction efficiency is below 100%, which is the case with our device, a depletion of CO₂ in the extracted air could occur. However, calculations reveal that ice of the Holocene period from Taylor Dome is clearly above the clathrate zone, and so this problem is not relevant.

Samples and chronology

The ice core from Taylor Dome (77° 48' S, 158° 43' E; elevation, 2,374 m above sea level; accumulation rate, 7 cm ice equivalent per year, ref. 21)—hereafter referred to as TD—is 554 m long and was drilled in 1993/94 by a US consortium. Due to the low annual mean temperature of −42 °C (ref. 22), the occurrence of melt layers is unlikely. Analyses of the isotopic composition of the ice (δD , a proxy for temperature) reveal that the glacial–interglacial transition is between 375 and 353 m depth²³. For the Holocene CO₂ record, a total of 417 samples from 69 different depth intervals were

measured in Bern. An additional 50 samples from 15 different depth levels were measured in San Diego for interlaboratory comparison. Furthermore, 13 samples were measured in San Diego for the $\delta^{13}C$ record.

The porous firn layer on the surface of an ice sheet is continuously exchanging air with the overlying atmosphere. Therefore, the air at the bubble close-off depth is younger than the surrounding ice. The present-day close-off depth at TD can be calculated using the model of Martinier *et al.*²⁴ and the density profile of TD²⁵; it is located at 72 m. In order to obtain an age of the air enclosed in bubbles,

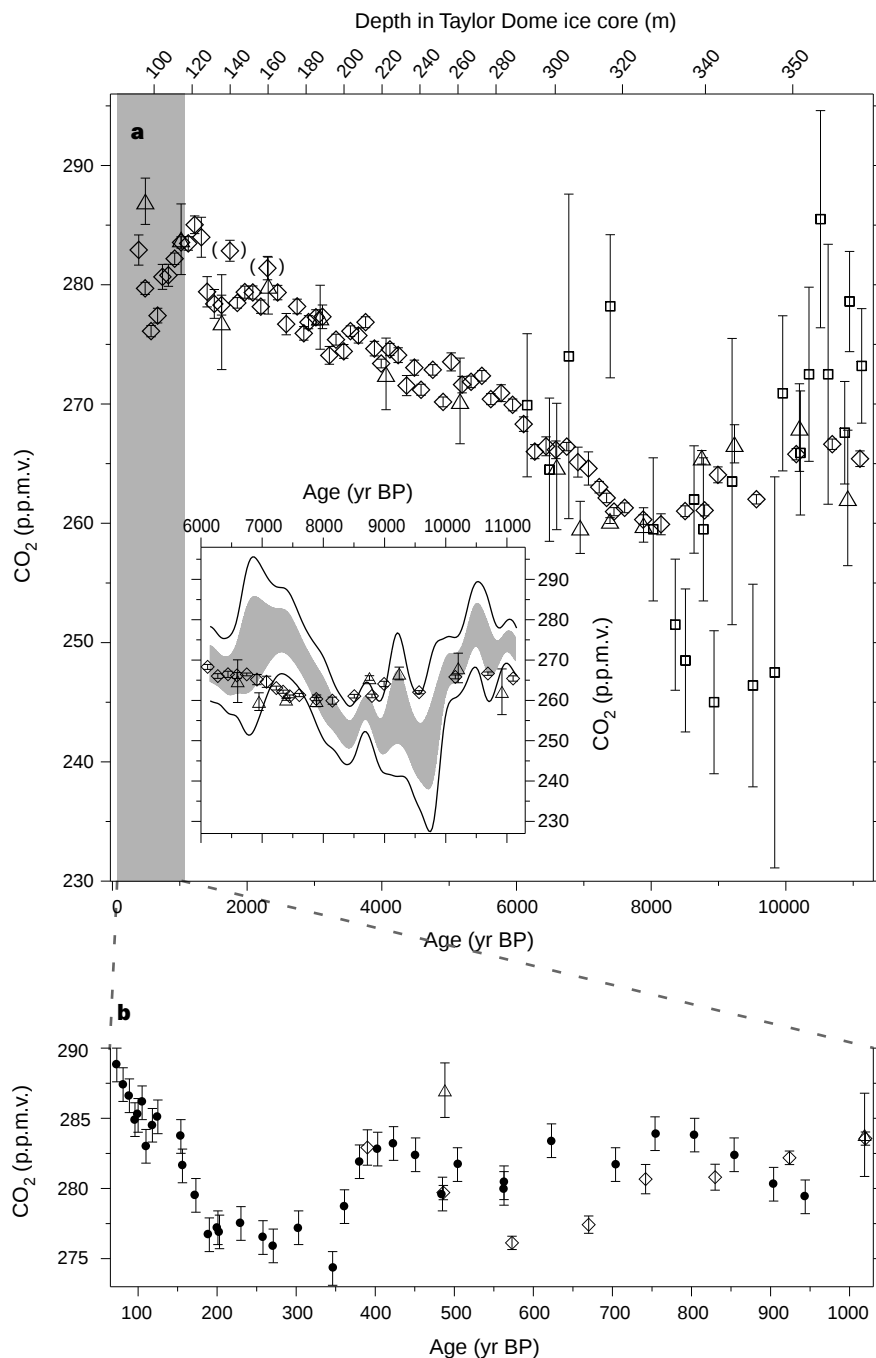


Figure 1 Mean CO₂ concentrations from ice cores. **a**, Open diamonds, mean of five to six samples from Taylor Dome ice core measured in Bern. Open triangles, mean of three to six samples measured in San Diego. Open squares, data from Byrd Station ice core⁵. Error bars, 1σ of the mean. The brackets indicate samples where natural artefacts cannot be excluded. Inset, as **a** but for the interval where the Byrd Station and Taylor Dome data overlap. Using a Monte Carlo method,

1,000 time series of the CO₂ record from Byrd Station were generated and filtered in order to take into account the different enclosure processes at Byrd Station and Taylor Dome, respectively. Shaded area, 1σ band; solid lines, 2σ band. **b**, Expanded-scale view of the data for the past millennium (shaded area in **a**). Open diamonds and triangles, mean CO₂ concentrations from Taylor Dome; dots, CO₂ concentrations from Law Dome⁴.

the CH₄ record from TD was synchronized with its well-dated GISP2 counterpart²⁶, and the age of each CO₂ sample linearly interpolated from the CH₄-based age scale. The uncertainty of the chronology (mainly due to the uncertainty of the synchronization) is <200 yr between 12–8 kyr BP; in this age range, distinct changes in the CH₄ concentration occur (the Younger Dryas event, and the 8,200 yr event) and the resolution of the CH₄ record from TD is high. From 8 to 1 kyr BP, the uncertainty increases to ± 500 yr owing to the low resolution and the relatively smaller variation of the CH₄ records (E. J. Brook, personal communication). For the past millennium, we estimate the uncertainty of the chronology at ~ 100 yr.

Millennial variations of atmospheric CO₂ and $\delta^{13}\text{C}$

The measured CO₂ concentrations from TD are plotted in Fig. 1a versus age and depth, together with the CO₂ record from Byrd Station, West Antarctica⁷. The agreement between the values measured in Bern and San Diego is generally good. The most remarkable features of the TD record are the decrease from 268 p.p.m.v. at 10.5 kyr BP to 260 p.p.m.v. at 8.2 kyr BP, and the monotonic increase over the following 7 kyr to 285 p.p.m.v. Our data is in accordance with previously published data of the past millennium from the ice core of Law Dome⁴ (Fig. 1b) and with data from the Vostok ice core⁷ (not shown), taking into account the uncertainties of the chronology and the concentrations (1.2 p.p.m.v. for Law Dome). On the other hand, the values from Byrd Station and TD differ considerably in the overlapping section (11–6 kyr BP) and require further consideration.

The CO₂ concentration in the Byrd Station record in the period 11–6 kyr BP increases to 285 ± 9 p.p.m.v. at 10.5 kyr BP and shows large concentration changes of up to 40 p.p.m.v. and a minimum concentration of 245 ± 6 p.p.m.v. In contrast, the record from TD is remarkably smooth. In each depth interval we measured six TD samples along a 0.09–0.15 m section of the core corresponding to one or two samples per annual layer. The standard deviations of the six samples are generally smaller than the analytical uncertainty (1.5 p.p.m.v.). In contrast, standard deviations for Byrd Station samples in the section younger than 11 kyr BP are significantly higher than the analytical uncertainty at the time that the older record was measured (3 p.p.m.v.)⁶. The clathrate-formation zone at Byrd is between 700 and 1000 m (a gas age of 6.5–10.5 kyr BP) as indicated by the bad core quality⁵, and coincides with the region where data from Byrd and TD differ. The high scatter of the Byrd record could be explained by a combination of natural artefacts, fractionation in the clathrate-formation zone, or post-coring effects due to the brittleness of the core. We conclude that the Byrd record in the time interval 6–11 kyr BP is much less reliable than the TD record.

We have therefore good evidence that the TD record represents the atmospheric CO₂ concentration with great reliability because (1) the scatter of the adjacent measurements is statistically in agreement with the analytical uncertainty, (2) no clathrates occur in the part of TD discussed here, and (3), the TD record is in general agreement with the sparser Vostok record⁷. (We note that the San Diego measurements show an increased scatter due to the higher analytical uncertainty, see Methods).

In the interval 11–6 kyr BP, the mean values of CO₂ concentration from the Byrd core show similar oscillations to the TD core, but with much larger amplitude. The question arises as to whether both records represent the evolution of atmospheric CO₂, and differ only because of the different enclosure characteristics at the two drilling sites. The enclosure process acts as a low-pass filter, and can be expressed by a normalized Gauss filter with a time constant of $\tau_{\text{BS}} = 27$ yr for Byrd Station²⁷ and $\tau_{\text{TD}} = 140$ yr for Taylor Dome, respectively. For a better comparison of the two records, the following procedure was carried out. Assuming that the data from Byrd Station represent the atmospheric CO₂ concentration within the error bars, we generated 1,000 time series of the existing data

using a Monte Carlo method. The expected synthetic TD record was obtained by filtering the time series in order to take into account the different enclosure processes. The resulting 1 σ and 2 σ bands yield the record within which the TD values would fall if the Byrd CO₂ data between 11 and 6 kyr BP were a true atmospheric record. This can be compared with the measured CO₂ from TD (Fig. 1a, inset). More TD data points than expected from normal distribution are outside the 1 σ and 2 σ bands. Thus the difference between the two records is probably not due to the different enclosure processes.

In summary, the salient feature of the TD CO₂ record—a decrease of 8 p.p.m.v. from 11 to 8 kyr BP, and a subsequent increase by 25 p.p.m.v. over the following 7 kyr (Fig. 2a)—is the atmospheric imprint of an evolving global carbon cycle.

The measured $\delta^{13}\text{C}$ values from TD show an increase from -6.6‰ at 11 kyr BP to -6.3‰ at 8 kyr BP, and then a gradual decrease to -6.5‰ at 1 kyr BP. There is no overlap between $\delta^{13}\text{C}$ from TD and Law Dome, but it seems that the TD values are in good agreement with those from Law Dome at 1 kyr BP ($-6.440\text{‰} \pm 0.013\text{‰}$)¹⁰. The data from TD at 11 kyr BP are in good agreement with the early Holocene average from Byrd ($-6.65\text{‰} \pm 0.07\text{‰}$)¹¹. Corrections made to the raw isotopic data include a correction for oxygen isotopes, a gravitational separation correction (using interpolated $\delta^{15}\text{N}$ data^{23,28}), and a correction for the daily (machine) offset of the air standard. An additional N₂O correction is based on measurements with mixtures of CO₂ and N₂O, from which we have calculated the magnitude of the correction as a function of N₂O/CO₂. We use the published values of N₂O concentration in air²⁹ as the raw data from which we calculate the corrections for N₂O in our samples. Measurements on different samples from the same depth, as well as measurements of standards, revealed an uncertainty of the $\delta^{13}\text{C}$ data of $\pm 0.085\text{‰}$, including fractionation effects during the extraction and the uncertainty of the $\delta^{15}\text{N}$ data.

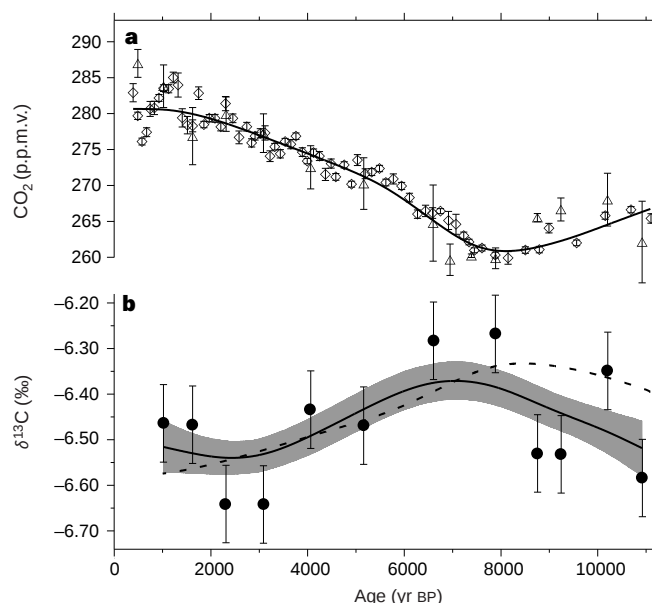


Figure 2 CO₂ concentrations and stable-isotope ratios, Taylor Dome. **a**, CO₂ concentrations. Open diamonds and triangles, mean CO₂ concentration. Error bars, 1 σ of the mean. Solid line, spline fit⁴⁸ of the CO₂ results used as input for the single and double deconvolution. This spline fit method acts as a low-pass filter; parameters were selected to obtain a cut-off period of $\sim 8,000$ yr which is consistent with the low resolution of the $\delta^{13}\text{C}$ record. **b**, Measured and calculated $\delta^{13}\text{C}$. Filled circles, $\delta^{13}\text{C}$ values. Error bars, estimate of the reproducibility of the $\delta^{13}\text{C}$ values. Solid line and shaded area, input values of $\delta^{13}\text{C}$ for the Monte Carlo simulation. Dashed line, $\delta^{13}\text{C}$ calculated with the single deconvolution³¹ (land-biosphere-only hypothesis, h1; see text).

Mechanisms of change

The main sources and sinks of atmospheric CO₂ on a millennial timescale are the land biosphere and the ocean. Atmospheric CO₂ concentrations and $\delta^{13}\text{C}$ are approximately in dynamical equilibrium with the surface ocean, as dictated by the distribution of dissolved inorganic carbon and ¹³C, alkalinity, temperature and salinity in the surface ocean³⁰. Changes in atmospheric CO₂ concentrations and $\delta^{13}\text{C}$ can be caused by changes in terrestrial biomass, in physical processes such as meltwater input, surface ocean warming, changes in air–sea gas exchange and ocean circulation, and in the marine biogeochemical cycles of organic matter and calcite (CaCO₃). Consequently, a suite of palaeoclimatic observations would be necessary to quantify the contribution of these individual processes to the observed atmospheric changes. However, budget calculations of C and ¹³C allow us to validate at least the consistency of explanations for the observed atmospheric CO₂ and $\delta^{13}\text{C}$ changes.

Inverse methods (referred to as single³¹ and double deconvolution¹³), based on a version of the Bern carbon-cycle model³², were applied to interpret quantitatively the observed CO₂ and $\delta^{13}\text{C}$ variations. As a minor modification, it is assumed that 70% of the carbon released by the land biota during the Holocene is absorbed by sediments on an exponential timescale of 5 kyr (ref. 33). The ocean part of the model includes two surface boxes, representing low- and high-latitude surface water masses. We note that the millennial timescale of the observed atmospheric variations is comparable to the timescale of ocean overturning and is several orders of magnitude longer than atmosphere–surface ocean exchange. Thus, the atmosphere–ocean system is always very close to equilibrium, and results are insensitive to the assumed surface-to-deep transport rates and air–sea exchange coefficients.

Our inverse modelling results presented below suggest that the observed atmospheric variations during the Holocene were driven by a combination of growth (11–7 kyr BP) and decay of terrestrial biomass, and by an increase in sea surface temperature (SST) of ~0.5 °C between 9 and 6 kyr BP. In addition, the slow re-equilibration between the ocean and sediment systems in the wake of the glacial–interglacial transition³⁴ may also have contributed.

We explored quantitatively four hypotheses, where the atmospheric variations are driven entirely by variation in the land biomass (h1), or by a combination of terrestrial changes plus variations in either SST (h2), the CaCO₃ cycle (h3) or the marine organic carbon cycle (h4). Furthermore, a simple estimate shows that meltwater input from ice-sheet remains had a marginal effect on atmospheric CO₂ and $\delta^{13}\text{C}$ during the Holocene. The input of melt water reduces sea surface salinity, alkalinity and dissolved inorganic carbon by dilution of the ocean water. Between 10.5 and 8.2 kyr BP, sea level rose ~26 m (ref. 35). Assuming a well-mixed ocean and chemically pure melt water (that is, a maximum dilution effect), the corresponding meltwater input would cause no change in atmospheric $\delta^{13}\text{C}$ and a decrease of the atmospheric CO₂ concentration of 2.8 p.p.m.v. (ref. 36), which is clearly less than observed.

Land biota. In the land-biota-only hypothesis (h1), the ocean reacts solely in a passive way to the Holocene atmospheric CO₂ variations. It is assumed that the carbon cycle was in equilibrium at 11 kyr BP, and that the physical and biological properties of the ocean remained unmodified during the Holocene. The carbon uptake of the ocean for slowly varying concentrations of atmospheric CO₂ is then dictated by its chemical capacity to absorb carbon released into the atmosphere–ocean system. The inverse method (single deconvolution³¹) is used for quantification. The net carbon release by the land biosphere then equals the observed rate of change in atmospheric CO₂ (time derivative of solid line in Fig. 2a) plus the modelled uptake by the ocean. The change in atmospheric $\delta^{13}\text{C}$ (dashed line in Fig. 2b) is calculated applying time-invariant isotopic fractionation of 18.7‰ (ref. 13). The comparison of measured and modelled $\delta^{13}\text{C}$ permits a first test of our hypothesis

(Fig. 2b). From 11 to 6 kyr BP, the calculated increase of 0.06‰ is much smaller than the measured increase of 0.3‰. On the other hand, there is a rather good agreement from about 6 to 1 kyr BP. This agreement implies that the ocean carbon cycle probably operated on a millennial timescale in a remarkably constant mode after 6 kyr BP. Short-term fluctuations induced CO₂ concentration perturbations of less than ±5 p.p.m.v. The calculated cumulative biospheric carbon release during 7–1 kyr BP is 260 GtC (not shown). We conclude that the observed increase of CO₂, and the $\delta^{13}\text{C}$ decrease, from 6 to 1 kyr BP can be explained consistently by terrestrial biosphere forcing only, but that additional mechanisms must be considered to explain the CO₂ and $\delta^{13}\text{C}$ changes between 11 and 6 kyr BP.

Sea surface temperature. A change of SST by 1 °C causes a change in the surface ocean's CO₂ partial pressure by 4.2% (ref. 36) which translates into an atmospheric change of similar magnitude³⁷. A SST-only model for the observed CO₂ evolution would require a gradual decrease of global mean SST from 10.5 to 8.2 kyr by 0.7 °C and then an increase of 2.3 °C during the following 7 kyr. However, this is not compatible with the $\delta^{13}\text{C}$ data. An increase in SST of 2.3 °C would correspond to an increase in the air–sea equilibrium fractionation factor, and thus in atmospheric $\delta^{13}\text{C}$, of about 0.25‰ (ref. 38). Instead, TD data show a decrease from 8 to 1 kyr BP. Thus, SST can not have been the only mechanism for the observed changes, although it could have contributed significantly.

Alkenone palaeothermometry³⁹ and temperature reconstructions from bore-hole measurements in Greenland ice⁴⁰ indicate a significant warming during the first part of the Holocene. To test the land biota–SST hypothesis (h2), we performed a double deconvolution¹³ where both CO₂ and $\delta^{13}\text{C}$ (solid lines in Fig. 2a, b) are used to calculate the two unknown net carbon fluxes into the ocean and into the terrestrial biosphere. Therefore, the assumption of a steady-state natural carbon cycle (as used in the single deconvolution) can be relaxed. SST is then calculated in the model by requiring consistency between simulated net air-to-sea carbon flux and simulated (very small) air–sea CO₂ partial-pressure difference. This means that changes in the sea-surface partial pressure of CO₂, as driven by changes in dissolved inorganic carbon and SST, are forced to be approximately equal to the atmospheric changes. An exponential relationship between partial pressure and SST, based on the thermodynamics of carbonate

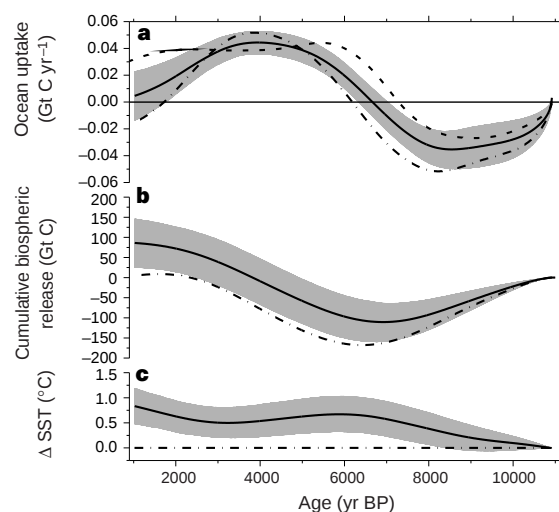


Figure 3 Comparison of our hypotheses. Dashed lines, land-biosphere-only hypothesis (h1). Solid lines, land biota–SST hypothesis (h2). The shaded areas indicate the 1 σ confidence interval of a Monte Carlo analysis, taking into account the uncertainty of the ice-core data. Dash-dotted lines, land biota–calcite hypothesis (h3). **a**, Ocean uptake. **b**, Cumulative biospheric release. **c**, Required change in SST.

chemistry³⁶, is applied. Air–sea fractionation factors are described as functions of SST³⁸. A Monte Carlo simulation¹³ including 2,000 model runs was performed to assess the uncertainties in the net fluxes introduced mainly by the uncertainty of the $\delta^{13}\text{C}$ data (Figs 2 and 3: best estimate, solid lines; shaded area, 1σ bands). SST (Fig. 3c) remained nearly constant during 11–9 kyr BP and after 6 kyr BP, whereas it increased by about 0.5 °C between 9 and 6 kyr BP, roughly in agreement with alkenone palaeothermometry reconstructions³⁹. Significant deviations between the single- (land-biota-only hypothesis, h1) and the double-deconvolution results for the combined land biota–SST hypothesis h2 are only found between 8 and 5.5 kyr BP (Fig. 3a, dashed line and shaded area). About one-third of the atmospheric $\delta^{13}\text{C}$ increase is explained by the warming of the ocean surface, while the rest is driven by terrestrial carbon uptake. A cumulative biospheric uptake (solid line/shaded area in Fig. 3b) of 110 ± 47 Gt C between 11 and 7 kyr, and a release of 195 ± 40 Gt C between 7 and 1 kyr, is obtained.

Marine biological cycle. It has been suggested that the ocean–sediment system continued to adjust towards a new equilibrium after the glacial–interglacial transition³⁴. The observed $\delta^{13}\text{C}$ changes cannot be due to a change in the marine calcite cycle only, however, because the isotopic ratio of biogenic calcite is very close to that of ocean surface water. Next, we investigate the land biota–calcite hypothesis (h3) by adjusting the partial pressure of surface-water CO_2 towards the atmospheric pressure as for h2, but without varying SST in the double deconvolution. Again, the atmospheric observations suggest that changes in the land biota alone were responsible for the variations before 9 kyr BP and after 6 kyr BP, as shown by the rather close agreement between the ocean uptake for the land-biota only case (h1; dashed line in Fig. 3a) and the land biota–calcite hypothesis (h3; dot-dashed line in Fig. 3a). For the period 9–6 kyr BP, a significant contribution from variations in the calcite cycle is required. The result is not consistent with the expectation of a system adjusting slowly towards a new equilibrium, in which the contribution by calcite changes to the observed trends would be largest at the beginning of the record. However, uncertainties due to the scarcity of $\delta^{13}\text{C}$ data are significant. Our results suggest that adjustments in the calcite cycle after the glacial–interglacial transition may have contributed to the overall atmospheric variations, but did not play the dominant role.

An increase of the export of organic matter from the ocean's surface layer to the deep sea would lead to a decrease of the concentration of atmospheric CO_2 and an increase in atmospheric $\delta^{13}\text{C}$. If the decrease of CO_2 of 8 p.p.m.v. from 10.5 to 8.2 kyr BP and the increase of 25 p.p.m.v. from 8.2 to 1 kyr BP were caused by changes in the marine organic carbon cycle only, then $\delta^{13}\text{C}$ would first increase by 0.08‰ and then decrease by 0.25‰ (ref. 11). This is not in quantitative agreement with the $\delta^{13}\text{C}$ measurements, as the required increase of $\delta^{13}\text{C}$ is too small by a factor of 3. We investigate a land biota–marine organic cycle hypothesis (h4) by adjusting the partial pressure of surface water CO_2 towards the atmospheric pressure (as for h2) by varying the export production of marine biota instead of SST in the double deconvolution. Net fluxes for this hypothesis are up to 0.12 Gt C yr^{−1} and are thus much higher than for hypotheses h1–h3. The close correlation between the export production and the terrestrial net flux, as suggested by hypothesis h4, implies a close phase relationship between the two. However, there is no indication that such a strong coupling exists.

Sensitivity. How sensitive are our results to model parameters? We have varied model parameters for our preferred land biota–SST hypothesis (h2). As discussed previously, the results are not sensitive to surface-to-deep ocean mixing and air–sea exchange coefficient. One remaining model parameter is the air–biota fractionation factor. We assumed that the released biogenic material originates from C3 plants, implying an air–biota fractionation factor of 18.7‰ (ref. 13). Changes in calculated sinks were small when this fractionation factor was set to 10‰, assuming that two-thirds of the

released biogenic material (of the order of 100 Gt C) originated from C4 plants⁴¹. We also varied the air–biota fractionation factor by $\pm 0.1\text{‰ kyr}^{-1}$ to account for possible transient changes in fractionation that affected all vegetation and soil pools (of the order of 2,000 Gt C). In both cases, we calculated significant deviations of the net carbon fluxes between the standard case (h2) and the sensitivity runs: $\sim 25\%$ from 11 to 7 kyr BP, and $\sim 40\%$ from 7 to 1 kyr BP. The resulting additional uncertainty of the cumulative biospheric release is ± 30 Gt C at 7 kyr BP, and ± 75 Gt C at 1 kyr BP. The additional uncertainty in the required change in SST is ± 0.2 °C at 7 kyr BP, and ± 0.5 °C at 1 kyr BP.

Discussion

The global carbon cycle has not been in steady state during the past 11 kyr. On the basis of our model estimates, we suggest that the observed variations of CO_2 and $\delta^{13}\text{C}$ over this period are caused by a combination of growth and decay of terrestrial biomass, and an increase in global mean SST, possibly with a contribution from the marine calcite cycle.

The biospheric uptake of carbon between 11 and 7 kyr BP is consistent with expectations based on vegetation regrowth and soil build-up on areas initially covered by ice sheets, as well as a climatic development towards the mid-Holocene optimum. The simulated uptake of 110 Gt C corresponds to roughly 20% of the estimated glacial–interglacial change in terrestrial storage of 500 Gt C (ref. 42). The cumulative biospheric release of 195 Gt C from 7 to 1 kyr BP could be due to a change from the warmer and wetter mid-Holocene climate to colder and drier conditions. Such changes in northern subtropical regions have been inferred from pollen, lake-level and CH_4 data^{43,44}. The changes of the vegetation types in northern Africa and the Arabian peninsula in the past 6.8 kyr have recently been reconstructed, indicating a development from steppe and savannah to desert⁴⁵. Using a biosphere model⁴⁶, we calculate carbon storage of 9.5×10^{-6} Gt C km^{−2} in warm grass/shrub, 11×10^{-6} Gt C km^{−2} in tropical dry forest/savannah, and 7.3×10^{-6} Gt C km^{−2} in desert. A difference of about 30 Gt C stored in this area between 6.8 kyr BP and today can be estimated, which is much less than that calculated with our double deconvolution procedure. If our hypothesis is correct, regions in addition to northern Africa and the Arabian peninsula should have contributed substantially to the changes in the terrestrial biosphere.

The rate of change of atmospheric CO_2 concentration over the Holocene is two orders of magnitude smaller than the anthropogenic CO_2 increase since industrialization. Understanding modifications of the distribution of biomes due to past and future climate change is becoming increasingly important, and could be investigated using models of the terrestrial biosphere. The Taylor Dome CO_2 and $\delta^{13}\text{C}$ records would serve as important checks for results derived from such models. □

Methods

Measurements of CO_2 (Bern). From each depth interval in the ice core, six samples (of size $2.5 \times 2.5 \times 1.5$ cm³, resulting in a depth resolution of 2.5 cm for most of the depth intervals and 1.5 cm for the rest) are cracked in an evacuated and cooled needle cracker. To measure the CO_2 concentration of the extracted gas, an infrared laser is tuned several times over the absorption line of a vibration–rotation transition of the CO_2 molecule. Calibration is routinely done using reference gases from the Scripps Institution of Oceanography (251.7 p.p.m.v., 321.06 p.p.m.v.). Measurements on bubble-free single-crystal ice samples, to which reference gas is added, yield an estimate of ± 1.5 p.p.m.v. for the analytical uncertainty of the device.

Measurement of CO_2 (San Diego). The procedure is described in refs 15 and 47. Three standards between 165 and 330 p.p.m.v. are run over the crushed ice for every three samples, to simulate the conditions in the procedure. The analytical uncertainty of a single CO_2 concentration measurement is ± 3 p.p.m.v.

Measurement of $\delta^{13}\text{C}$. The procedure at San Diego is as follows.

Approximately 200 g of carefully trimmed ice is crushed under vacuum in a rotary, inwardly spiked cylinder in a -26°C freezer for 30 min. The CO_2 content of the air thus liberated from the ice is extracted on a glass vacuum rack by passing it through a -90°C acetone/liquid nitrogen (LN) trap to remove water and two -196°C LN traps to trap the CO_2 . The CO_2 is transferred from the first -196°C trap to the second by warming the first to -90°C , then into a Pyrex sample tube cooled to -196°C which is flame sealed. This sample is analysed on a mass spectrometer (VG PRISM II IRMS) for isotopic composition of C and O. $\delta^{13}\text{C} = [(^{13}\text{C}/^{12}\text{C})_{\text{sample}}/(^{13}\text{C}/^{12}\text{C})_{\text{standard}}] - 1$, referred to the marine carbonate standard PDB.

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An outburst of relativistic particles from the soft γ -ray repeater SGR1900+14

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Soft γ -ray repeaters (SGRs) are transient sources of high-energy photons, whose brief emissions are thought to arise from young¹ and highly magnetized^{2–6} neutron stars. The exact cause of these outbursts, and the nature of the energy-loss mechanism that powers them, remain unknown. Here we report the discovery of a fading radio source within the X-ray error box of SGR1900+14. We argue that the radio source is a short-lived cloud of ionized gas, powered by relativistic particles ejected at the time of the intense burst of high-energy photons in late August 1998 (this period of activity also included an extremely energetic burst of γ -rays⁷ on 27 August). As the radio photons are not beamed, our observations allow us to constrain the energy released in the form of particles ejected during the burst. Moreover, the astrometrical precision of radio observations enable us to determine the exact position of the source to very high accuracy.

Until recently SGR1900+14 was routinely labelled as the least prolific of the SGRs, with only three bursts⁸ in 1979 and another three⁹ in 1992. We investigated the relatively crude localization obtained from the 1979 activity and suggested¹⁰ that this SGR is associated with the supernova remnant (SNR) G42.8+0.6 (see also ref. 11). Our observations with the Very Large Array (VLA) showed that G42.8+0.6 was a typical shell-type object. Furthermore, we suggested¹⁰ that the X-ray source RX J190717+0919.3 located within the tighter 1994 localization¹² was the quiescent counterpart of the SGR. Subsequent observations with the High Resolution Imager (HRI) on board the Rosat satellite resulted in a tighter localization¹³ of the source.

We began a monitoring program of RX J190717+0919.3 at the VLA, motivated by the notion that the high-energy bursts are probably accompanied by bursts of energetic particles which could power a synchrotron nebula, a mini-“plerion”. Our initial observations of the X-ray source showed no strong radio emission at, or close to, the position of RX J190717+0919.3. In Table 1 we list the results from our VLA monitoring study of RX J190717+0919.3.

Our first VLA observation after the bright burst of 27 August was made on 3 September. As can be seen from Fig. 1b, a new source is apparent within the localization circle of RX J190717+0919.3. This radio source was not seen in earlier VLA images obtained from data obtained at the same frequency and similar resolution and sensitivity; see Fig. 1a. The probability of finding a steady background radio source by chance¹⁴ in this area is 10^{-3} . The unusual time variability reduces the probability further still, but by an unknown factor. The radio source is unresolved and we place an upper limit to the angular diameter of $0.8''$. The precise position of the transient source (epoch J2000) is right ascension 19 h 07 min 14.33 s, declination +09° 19' 20.1", with an uncertainty of $0.15''$ in each coordinate.

Following the detection on 3 September, the source underwent a rapid, monotonic decay and is no longer detectable; see Table 1. The decaying portion of the curve can be fitted to a power law (flux $\propto t^\delta$ with $\delta = -2.6 \pm 1.5$; here t is time since the burst of 27 August; see Fig. 2b). On 8 September observations were also made at 1.43 GHz and 4.86 GHz, yielding a spectral index $\alpha = -0.74 \pm 0.15$ (where the flux at frequency ν , $S_\nu \propto \nu^\alpha$); see Fig. 2a. No linear or circular

polarization is detected but due to the faintness of the source, the limits are not restrictive; the percentage polarization is $<30\%$.

Radio sources which show such transient behaviour on this timescale are quite rare in the sky. Furthermore, the source lies in the Ulysses–CGRO triangulation⁶, which has a total area of 17 arcmin^2 . We suggest that the transient radio source VLA J190714.3+091920 is powered by the heightened burst activity from SGR1900+14 in late August, which included the intense burst of 27 August. If we are correct, then we have localized SGR1900+14 to sub-arcsecond accuracy. Hurley *et al.*⁶ report 5.17-s X-ray pulsations from the vicinity of RX J190717+0919.3. Such long-period pulsations⁵ are also seen from the quiescent¹⁵ counterpart of SGR1806–20 and in the bright burst⁴ from SGR0526–66. Thus RX J190717+0919.3 is very likely to be the X-ray counterpart of SGR1900+14.

SGR1806–20 offers an excellent ‘archetype’ against which we now interpret our radio observations of SGR1900+14. To start with, both SGRs are associated with SNRs: SGR1806–20 is embedded in the plerionic SNR G10.0–0.3 (refs 16–18) whereas SGR1900+14 is found just outside G42.8+0.6 (ref. 10). The intense bursts, the quiescent X-ray counterparts, the long-period pulsations and the associated SNRs have been interpreted in the framework of the magnetar model^{2,3}. Magnetars are highly magnetized neutron stars with dipole field strengths of 10^{14} – 10^{15} G, considerably larger than those of radio pulsars. The ultimate source of energy for the bursts and the quiescent emission comes from the decay of the magnetic field. The non-thermal quiescent X-ray emission and the highly suggestive radio images^{17,18} of SGR1806–20 provide compelling evidence for a steady particle wind from the SGR. The “nested” appearance¹⁶ of G10.0–0.3 is best explained¹⁷ as being powered by an episodic injection of energy from the underlying SGR. Thus G10.0–0.3 is powered both by a steady and an episodic power source.

VLA J190714.3+091920 shares characteristics with G10.0–0.3. Both have low brightness temperatures (a lower limit of 10 K is derived for SGR1900+14 from the upper limit on the angular size of $0.8''$) and unusual (for a plerion) non-thermal radio spectra indices (-0.6 and -0.74). The main distinction is that G10.0–0.3 is bright and long-lived whereas VLA J190714.3+091920 is short-lived and fainter. We attribute these differences to (1) the low-pressure environment surrounding SGR1900+14 and (2) the lower energy loss and activity of SGR1900+14 as compared to that of SGR1806–20. The radio nebula around SGR1806–20 is bright because this prolific SGR (with implied large energy loss) is embedded in a high-pressure environment, the SNR G10.0–0.3. Thus the particle wind emanating from SGR1806–20 is contained, thereby accounting for

Table 1 VLA observations of RX J190717+0919.3

Date (UT)	Frequency (GHz)	Flux (μJy)	r.m.s. (μJy)
1994 Oct. 05.00	1.43		110
1994 Oct. 05.01	8.41		30
1995 Dec. 14.80	8.41		33
1995 Dec. 21.96	8.41		55
1995 Dec. 26.81	1.43		125
1995 Dec. 26.82	4.59		44
1998 Jun. 25.25	8.46		46
1998 Sep. 03.12	8.46	285	38
1998 Sep. 06.26	8.46	315	52
1998 Sep. 08.07	1.43	745	110
1998 Sep. 08.08	4.86	300	33
1998 Sep. 08.09	8.46	206	26
1998 Sep. 14.24	8.46	70	43
1998 Oct. 01.06	8.46		25
1998 Oct. 11.12	1.43		70

Columns (left to right) show the UT date of the observation, the observing frequency, the flux density of the source if it was detected, and the r.m.s. noise in the image. All observations were made with a bandwidth of 100 MHz at each frequency. Antenna phase calibration was accomplished using extragalactic radio sources with well-known positions near SGR1900+14. The absolute flux scale (accurate to better than $\pm 2\%$) at each epoch was fixed by short observations of the radio sources 3C48, 3C147, or 3C286. The angular resolution at 8.46 GHz was ~ 0.8 – 1.2 arcsec, increasing linearly with decreasing frequency.

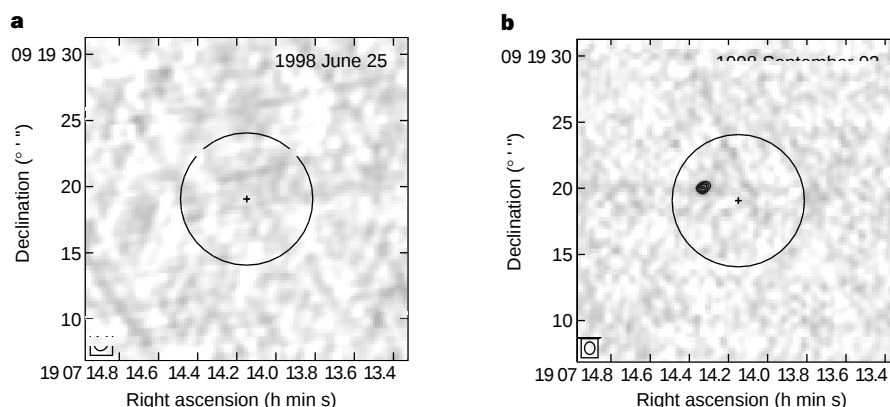


Figure 1 Images made at 8.46 GHz of the field around RX J190717+0919.3, a source proposed¹⁰ as the X-ray counterpart of SGR1900+14. Although ref. 13 does not discuss the size of the error circle, our assumed value of 10-arcsec radius is an exceedingly conservative value. The “+” sign is the nominal location of RX

J190717+0919.3; coordinates are epoch J2000. The 25 June image (a) does not show any evidence for a radio source to a 2σ level of $92 \mu\text{Jy}$. On 3 September (b) there is an unresolved $300\text{-}\mu\text{Jy}$ radio source VLA J190714.3+091920 within the X-ray localization. This source faded to an undetectable level by 1 October.

the high surface brightness. In contrast, SGR1900+14 is not as prolific and lies outside G42.8+0.6. Thus the particle wind from SGR1900+14 is confined only by the ram pressure from the motion of SGR1900+14 through the interstellar medium. This weaker confinement does not allow for the build-up of a plerion, thereby resulting in a weaker plerion.

Accepting our reasoning that VLA J190714.3+091920 is a synchrotron-emitting nebula, we apply the synchrotron model¹⁹ to derive physical parameters. There are two main unknowns: the distance and angular size of the source. On general grounds, the distance to SGR1900+14 is roughly 10 kpc, given that it is located in the inner Galaxy. However, if SGR1900+14 is associated with G42.8+0.6 then a distance of 5 kpc is probably reasonable¹⁰. The minimum energy of the nebula (integrating the spectrum shown in Fig. 2a from 10^7 Hz to 10^{11} Hz) and the equipartition magnetic field strength are then respectively $U_{\min} = 3 \times 10^{42} d_5^{1/7} \theta_4^{9/7}$ erg and $B_{\min} = 0.55 d_5^{-2/7} \theta_4^{-6/7}$ mG; here the distance is $5 d_5$ kpc and the angular radius of the nebula is $\theta = 0.4 \theta_4$ arcsec.

Using the formulation of Scott and Readhead²⁰, we obtain a robust estimate of the angular size of the source, the so-called

“equipartition radius”, $\theta_{\text{eq}} = 165 d_5^{-1/17} S_p^{8/17} \nu_p^{-1.07} \mu\text{as}$ where ν_p is the peak of the synchrotron spectrum (in GHz) and S_p the corresponding flux (in mJy). The total energy of a source of size θ is $U = 1/2 U_{\text{eq}} \eta^{11} (1 + \eta^{-17})$ where $\eta = \theta/\theta_{\text{eq}}$ and U_{eq} is the minimum energy of a source with an angular radius of θ_{eq} . Small deviations from θ_{eq} result in steep energy demands, which is why most sources have angular sizes quite close to θ_{eq} . Unfortunately, we do not have sufficient wavelength coverage to see the true synchrotron peak. Instead we use the lowest-frequency data (1.43 GHz) on 8 September to obtain a true lower limit $\theta > \theta_a \approx 100 \mu\text{as}$. Using this angular radius results in $U_a \approx 7 \times 10^{37}$ erg and $B_a \approx 0.7$ G. The radiative decay timescale for the electrons responsible for the 8-GHz emission is then about 2 years.

Our incomplete knowledge of the true angular size of VLA J190714.3+091920 does not allow us to pinpoint the region from which the radio electrons originate and to infer the total particle energy and the magnetic field. We have two limits for θ , and both limits are viable. The strength of the magnetic field of a 6×10^{14} G magnetar rotating at 5.16 s (ref. 6) at the edge of the radio nebula of angular radius θ is $B_M \approx 0.13 (\theta/\theta_a)^{-1} d_5^{-1}$ G, comparable to B_a if

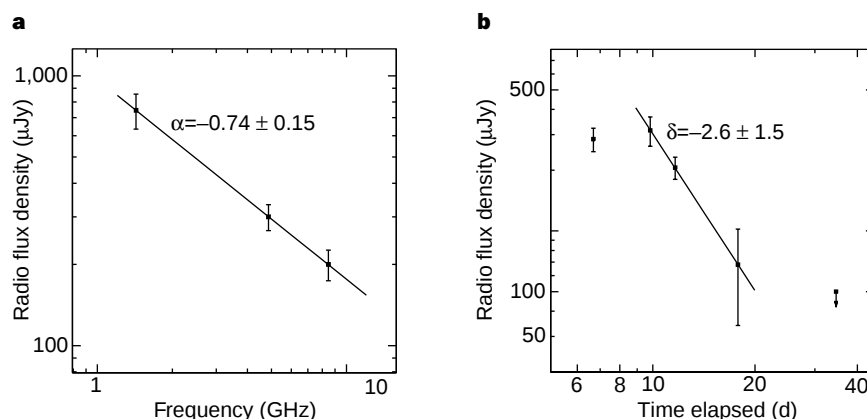


Figure 2 Data from the fading radio source. **a**, The spectrum of the radio transient on 1998 September 8 between 1 and 10 GHz. A weighted least-squares fit to these data gives a spectral slope $\alpha = -0.74 \pm 0.15$. **b**, The light curve at 8.46 GHz from 1998 September 3 to October 10. As the exact time for the onset of the radio

emission is unknown, the horizontal axis is drawn assuming that it originated at the same time as the γ -ray burst of August 27.432 UT. A weighted least-squares fit to the declining portion of the light curves gives a power-law slope of $\delta = -2.6 \pm 1.5$.

$\theta \approx \theta_a$. In the other extreme, when $\theta = 0.4$ arcsec, the magnetic field from the magnetar would be negligible, but the inferred field strength can be easily explained as arising from compression of the ambient field by the bow shock. The mean expansion speed of the nebula is $3.5 \times 10^{10} d_5 \theta_{10}^{-1} \text{ cm s}^{-1}$ where t_{10} is the age of the nebula in units of 10 days. Thus in the limit of $\theta = 0.4$ arcsec the nebula would have expanded relativistically. This is possible if previous outbursts have swept up the ambient gas. In this limit, U_{min} is comparable with the isotropic burst energy of $3 \times 10^{42} d_5^2 \text{ erg}$, as estimated from the fluence of the 27 August burst ($\sim 10^{-3} \text{ erg cm}^{-2}$; M. Feroci, personal communication). In either case, it is clear that nebular expansion accounts for the rapidly decaying radio emission. Thus the above energy estimates (derived from observations 7–10 days after the burst) need to be revised upwards to obtain the initial release of energy.

In the future, the availability of broad-band observations and/or direct measurement of the size of the nebula should enable us to estimate directly the energy of the burst in particles (without the uncertainties of beaming that bedevil estimates from γ -ray data). Equally important are accurate measurements of the average particle luminosity, as particle-aided spindown can substantially modify estimates for the magnetic field and the characteristic age of a neutron star²¹. These estimates, to our knowledge, are unobtainable in any other fashion. In those cases where the SGR is immersed in a high-pressure region (for example, SGR1806–20), we are able to trace the entire history of energy loss from the magnetar. The sub-arcsecond localization presented here should greatly help the identification of possible stellar counterparts of this SGR (as was done for SGR1806–20; refs 22, 23). □

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Rotation rates of Kuiper-belt objects from their light curves

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Very little is known about the physical properties of Kuiper-belt objects¹, due to their relatively small size and large distance from the Earth. For example, a Kuiper-belt object with a diameter of 300 km at a typical distance of ~ 30 AU would subtend an angle of only 0.014 arcsec. It is therefore possible to investigate their surface markings, shapes and rotational properties only through variations in the light that they reflect (their light curves). Here we report a survey of optical light curves from Kuiper-belt objects. Variations are observed only for the faintest objects in the survey. We can rule out eclipsing binary objects and variations in the surface markings as the origin of these light curves, suggesting that the observed variations are due to the rotation of irregularly shaped objects. Irregular shapes may be limited to the smallest Kuiper-belt objects because the material strength in their inner regions is sufficient to maintain the shape against the weight of the overlying material. If, however, all of the objects in our survey are of essentially the same size, then the intrinsically faintest ones may be composed of a stronger and darker material than the brighter ones.

Because Kuiper-belt objects (KBOs) are too faint for thermal infrared observations with a high signal-to-noise ratio, visible and near-infrared photometry seem to be the only way to learn anything about their diameters, shapes and surface markings. The diameter, D (in kilometres), of a KBO can be obtained from the definition of albedo, p :

$$p\Phi D^2 = 9 \times 10^{16} r^2 \Delta^{0.4(m_\odot - V)} \quad (1)$$

where Φ is the phase function², r is the heliocentric distance (AU), Δ is the geocentric distance (AU), $m_\odot$ is the apparent V-band solar magnitude (–26.74), and V is the apparent V-band magnitude of the KBO. The diameters that we derive below assume an albedo similar to that of the progeny of the KBOs—the nuclei of short-period comets. Such nuclei have $p = 0.04$, implying a surface as black as charcoal.

The shape or surface markings of a KBO can be derived from periodic variations in its brightness. In the case of a spherical KBO with light and dark surface markings (for example, with one hemisphere darker on average than the other), each rotation of the KBO results in one minimum and one maximum in the light curve. If the KBO has a uniform surface albedo, but an elongated shape, rotation of the object results in a periodic variation of the projected cross-sectional area. Each rotation of the elongated object results in two minima and two maxima in the light curve. The amplitude of the light curve, Δm , is related to the major axis, a , and the intermediate axis, b , of a triaxial object by the equation:

$$\Delta m = 2.5 \log \frac{a}{b} \quad (2)$$

where we have assumed that the axis of rotation is perpendicular to the line of sight (an 'equator-on' aspect) and lies along the short axis, c , of the body. The determination of the orientation of the rotation axis of a KBO from photometry would require data from about one-quarter of a KBO revolution about the Sun, about 40 years for a KBO at 30 AU. Such a determination is beyond the scope of this survey and therefore requires our simplifying assumption.

Our KBO survey uses Harris V (550 nm) and R (650 nm) 5×5 inch glass filters in front of a $1,200 \times 800$ pixel charge-coupled-device (CCD) camera at the $f/9$ Cassegrain focus of the Steward Observatory 2.3-m telescope on Kitt Peak, Arizona. The results presented here and in our KBO colour survey³⁻⁶ use the same data sets; the details of our observing and data-reduction procedures are contained in these earlier reports. We mention here that our aperture correction technique not only minimizes the sky noise in our measurements, but also minimizes errors due to contamination by faint galaxies and stars. An estimate of the effects due to contamination by faint background objects can be found by looking at the number-magnitude counts^{7,8} of faint stars and galaxies made to R -band magnitude $R = 25$ at relatively high Galactic latitudes, where many of the known KBOs are found. Extrapolating the number-magnitude relation to $R = 26$ mag, we find that the number of galaxies per degree² is larger than the number of stars per degree², and that there are about 2.5×10^5 galaxies per degree² between $R = 23$ mag and $R = 26$ mag; this corresponds to ~ 0.02 galaxy per arcsec². By using an aperture three pixels in radius for our KBO measurements, we greatly decrease our chances of including an 'unseen' (fainter than $R = 23$ mag) faint galaxy. The chance of finding a galaxy between $R = 23$ mag and $R = 26$ mag in our small aperture are 1 in 20.

Our V-band photometry is summarized in columns 2 and 3 of Table 1. An important aspect of our programme is that our photometry is consistent over intervals of hours, days and years. We find that 25 V-band magnitude measurements of the KBO 1996 TL66 taken over a time interval of seven days yield a standard deviation about the mean of 0.03 mag (Table 1). We obtain a similar consistency for 1996 TO66. Individual V-band magnitudes of the KBO 1993 SC agree at the 0.06-mag level over intervals of hours and days⁶. Once we correct for differences in distances and phase angle using a slope parameter of 0.15, we find that our V-band magnitudes for 1993 SC and 1994 TB are consistent at the 0.07-mag level over an interval of nearly a year and two years, respectively. Such repeatability in our photometry supports our hypothesis that contamination by faint stars and galaxies is not an important source of error in our KBO measurements.

We now consider how our photometry compares with that of other investigators. As there is little agreement between investigators regarding the $B - V$ and $V - R$ colours of KBOs⁴, we will confine our comparison to V-band magnitudes. First, we find that Jewitt and Luu⁹ observed the brightest KBOs (1996 TS66, 1996 TP66, 1996 TO66 and 1996 TL66) on 23 and 24 September 1997, about a week

before our measurements. For three of the four KBOs, our V-band magnitudes (Table 1) and their magnitudes differ by ≤ 0.05 mag, a remarkably good agreement. For an unknown reason, our colours and their colours for these same objects exhibit far less agreement. Second, we consider studies of the Centaur, 5145 Pholus. Buie and Bus¹⁰ and Davies *et al.*¹¹ obtain absolute magnitudes (brightness at unit heliocentric and geocentric distance and 0° phase angle), H_V , of 7.645 ± 0.011 and 7.63 ± 0.02 , respectively, for 5145 Pholus. We obtain $H_V = 7.63 \pm 0.03$ mag for 5145 Pholus, again very good agreement. Last, Davies *et al.* find good agreement between our V-band magnitudes and $V - R$ colours and their V-band magnitudes and $V - R$ colours for the Centaurs in their study.

There are 12 objects in Table 1 that have sufficient temporal coverage for use to measure, or set limits on, the amplitudes of light curves (see column 6 of Table 1). Of the KBOs, only 1995 QY9, 1994 VK8 and 1994 TB (November 1995 and October 1997) exhibit detectable brightness variations (Fig. 1). We have analysed the light curves in Fig. 1 using the techniques of phase dispersion minimization¹² as well as a phasing of the data with every possible period between 1 and 10 hours. We find that 1995 QY9 has a best period of ~ 3.5 hours, and a viable range of periods that extends between 3.3 and 3.7 hours. For 1994 VK8, we find possible light-curve periods of 3.9, 4.3, 4.7 and 5.2 hours. In the case of 1994 TB, we find that possible periods of 3.0 and 3.5 hours fit both the 1995 and 1997 data.

These periods are so short that they eliminate the rotation of essentially spherical objects with light and dark surface markings (for example, one hemisphere darker on average than the other) as the mechanism responsible for the light curves. Applying Newton's second law of motion and the law of gravitation allows us to calculate the period, P_{crit} , at which a KBO rotates so fast that it will throw material off its equator and hence be unlikely to retain a regolith or even to form in the first place by accretion:

$$P_{\text{crit}} = \left(\frac{3\pi}{G\rho} \right)^{1/2} \quad (3)$$

where ρ is the density. If we assume $\rho = 1 \text{ g cm}^{-3}$, we obtain $P_{\text{crit}} = 3.3$ hours. Our measured periods are slightly below, at, or slightly above, the critical period, making it unlikely that light and dark surface markings are responsible for the light curves.

The short measured periods make it unlikely that eclipsing binary KBOs are responsible for the light curves. The shortest period allowed for a binary KBO system corresponds to a separation

Table 1 Properties of KBOs

Object	V (mag)	σ (mag)	n	H_V (mag)	Δm (mag)	D (km)	a/b	UT Date
1997 CU26	18.56	0.02	2	6.75		300		8 Oct. 1997
1997 CS29	22.11	0.11	12	5.52	≤ 0.22	530	≤ 1.22	6, 9, 10 Oct. 1997
1996 TS66	22.47	0.08	7	6.50	≤ 0.16	340	≤ 1.16	5 Oct. 1997
1996 TQ66	23.12	0.11	10	7.69	≤ 0.22	200	≤ 1.22	4 Oct. 1997
1996 TP66	21.64	0.06	16	7.39	≤ 0.12	220	≤ 1.12	3-5 Oct. 1997
1996 TO66	21.38	0.05	13	4.75	≤ 0.10	760	≤ 1.10	3, 4, 6 Oct. 1997
1996 TL66	20.91	0.03	25	5.40	≤ 0.06	560	≤ 1.06	2, 9 Oct. 1997
1996 RQ20	22.94	0.07	7	6.99		270		10 Oct. 1997
1995 QY9	22.88		11	8.07	0.60	160	1.74	10 Oct. 1997
1995 HM5	23.4	0.1	3	8.30		150		8 Apr. 1997
1995 GO	20.12		15	9.19	0.34	100	1.37	14 Apr. 1996
1995 DW2	22.24	0.02	7	9.53	≤ 0.04	84	≤ 1.04	7 Apr. 1997
1994 VK8	24.05		15	7.53	0.42	210	1.47	24, 26 Jan. 1998
1994 TB	22.91		12	8.04	0.26	166	1.27	7 Oct. 1997
	23.20		12	8.11	0.34	161	1.37	24-27 Nov. 1995
1994 JR1	22.9	0.1	5	7.35		230		7, 8 Apr. 1997
1993 SC	22.74	0.02	4	7.23	≤ 0.04	240	≤ 1.04	24-27 Nov. 1995
	22.67	0.06	8	7.30	≤ 0.12	230	≤ 1.12	8 Oct. 1996
Nessus	20.84	0.04	2	9.52		80		14 Apr. 1996
Pholus	18.46	0.05	3	7.63	0.15 ^a	200		27 Nov. 1995

Meaning of symbols is as follows. V , Average V-band magnitude; σ , standard deviation about the average; n , number of images averaged; H_V , absolute magnitude; Δm , light-curve amplitude (objects without entries do not have sufficient temporal coverage to measure or set an upper limit on an amplitude); D , diameter, assuming a cometary albedo of 0.04; a/b , major-to-intermediate axial ratio, assuming that the axis of rotation is perpendicular to our line of sight.

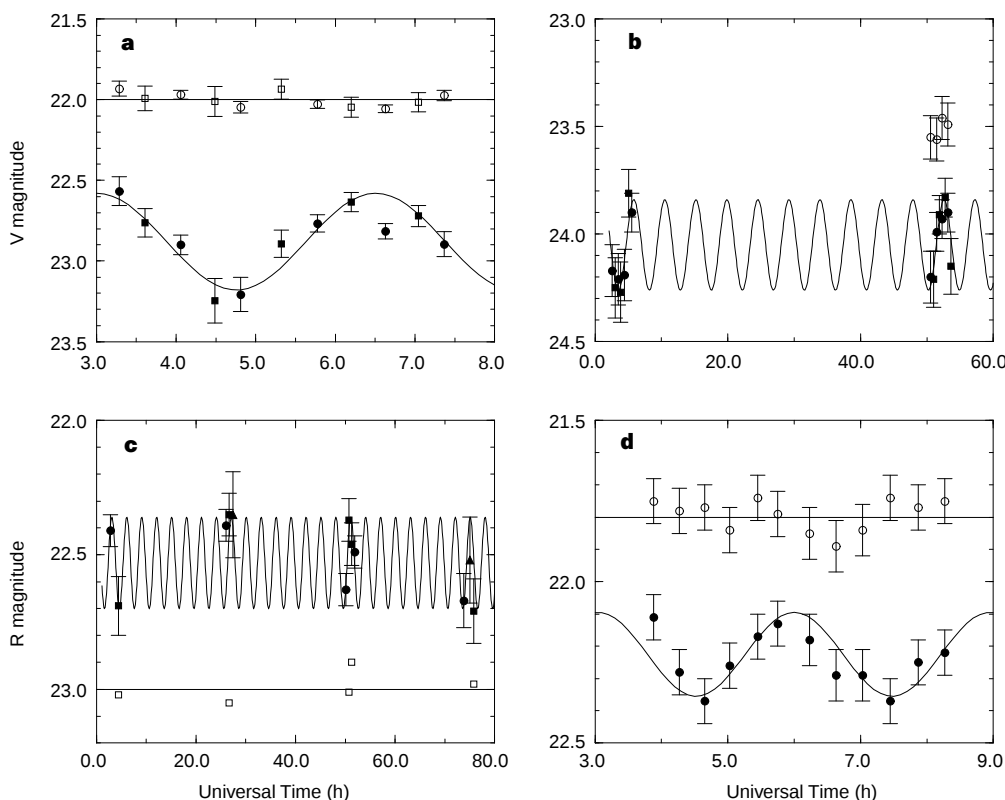


Figure 1 Light curves for Kuiper-belt objects (filled symbols) and faint field stars (open symbols). Measurements through the V and R filters are plotted as squares and circles, respectively. Conversions between V-band and R-band magnitudes are accomplished with the $V - R$ colours of the objects. All error bars are $\pm 1\sigma$ and are a measure of the sky noise. All sine curves represent possible periods and amplitudes obtained from our phase dispersion minimization and phasing techniques. **a**, V magnitudes for 1995 QY9 and a faint field star. The data from the star have been moved upwards on the figure for clarity; the actual average V magnitude and standard deviation of the star are 22.46 and 0.04, respectively.

The sine curve has a period of 3.5 h and an amplitude of 0.60 mag. **b**, V magnitudes for the KBO 1994 VK8 and a faint field star. The sine curve has a period of 4.7 h and an amplitude of 0.42 mag. **c**, R magnitudes obtained between 24 and 27 November 1995 for the KBO 1994 TB. The sine curve has a period of 3.0 h and an amplitude of 0.34 mag. **d**, R magnitudes obtained on 7 October 1997 for the KBO 1994 TB and a faint star. The data from the faint field star have been moved upwards for clarity; the actual R magnitude of the star is 22.19. The sine curve has a period of 3.0 h and an amplitude of 0.26 mag.

distance equal to the sum of the radii of the KBOs. If we assume similar sizes and densities for the two KBOs, then application of Newton's second law and the law of gravitation yields:

$$P_{\text{binary}} = \left(\frac{12\pi}{G\rho} \right)^{\frac{1}{2}} \quad (4)$$

If we assume a density of 1 g cm^{-3} , we obtain a minimum period of revolution for the binary of 6.6 hours. Because a complete cycle of the binary corresponds to two minima and two maxima in the light curve, we must double our KBO light-curve periods for comparison with the 6.6-hour minimum period for the binary. Once again, our measured periods are slightly below, at, or slightly above, the binary limit, making it unlikely that eclipsing binaries are responsible for the light curves.

Now that we have eliminated surface markings and binaries as mechanisms, it seems that our light curves are due to the rotation of irregularly shaped bodies. On doubling the light-curve periods, we find rotation periods of ~ 7.0 hours for 1995 QY9, possible periods of 7.8, 8.6, 9.4 and 10.4 hours for 1994 VK8, and 6.0 and 7.0 hours for 1994 TB. As a comparison, a histogram of rotation periods of asteroids with $125 \leq D \leq 200 \text{ km}$ has a peak at 12 hours (ref. 13).

We have also analysed the amplitudes of the light curves. A plot of the light-curve amplitude, and the 2σ upper limit for an amplitude, against absolute visual magnitude (H_V) for the 12 KBOs is shown in Fig. 2. A clear pattern can be seen in this figure: the intrinsically

brightest objects show no evidence of any significant brightness variation, whereas the intrinsically faintest objects do show such a variation.

The trend in Fig. 2 has two possible explanations. First, the KBOs in our sample could all have the same albedo: the difference in intrinsic brightness among the objects would then be due to differences in size. In the central region of a small KBO, the material strength exceeds the weight exerted by the overlying material. A small KBO can therefore be strong enough to retain any irregularity in its shape. In the central region of a very large KBO, on the other hand, the overlying weight of material exceeds the material strength, and crushes any irregularities in shape. Large KBOs must therefore take on a spherical shape. If we consider the diameter at which the transition between irregular and spherical KBOs takes place, D_t , we can estimate the strength of KBO material. If we assume a constant density throughout a KBO, then application of the equation of hydrostatic equilibrium at a radial distance of $0.8R$ from the KBO centre yields an expression for the material strength:

$$S = 1.29 \times 10^{-8} D_t^2 \rho^2 \quad (5)$$

where S is in dyn cm^{-2} and D_t is in cm. We chose a radius of $0.8R$ because half the mass of the KBO is inside such a radius, and the mechanical failure of ice at such a radius will probably manifest itself in terms of topographic relaxation in shape. An examination of column 7 in Table 1 suggests that the transition diameter occurs

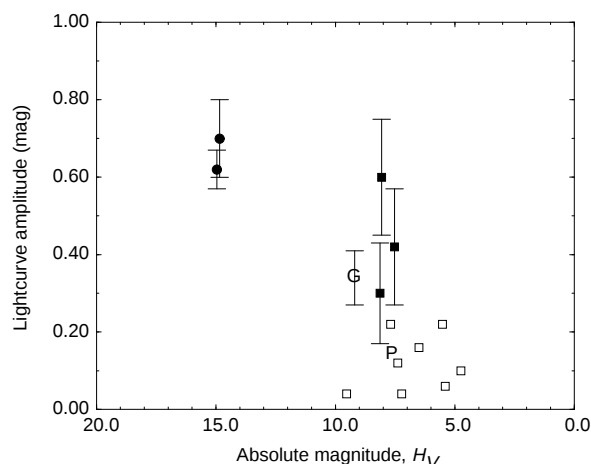


Figure 2 A plot of the light-curve amplitude versus the absolute magnitude for the 12 objects in our survey with good temporal coverage. The open squares and filled squares represent upper limits and Δm detections, respectively. The filled circles represent Δm detections for the bare nuclei of short-period comets^{19,20}. The symbols P and G represent the Centaurs 5145 Pholus¹⁰ and 1995 GO^{31,21}. A pattern is clearly seen; intrinsically faint KBOs exhibit light curves.

around 250 km, assuming an albedo of 0.04. If we assume a density of 1 g cm^{-3} , we obtain a material strength of $8 \times 10^6 \text{ dyn cm}^{-2}$, a value smaller than that of a bulk ice in a laboratory¹⁴, $2 \times 10^7 \text{ dyn cm}^{-2}$. Larger objects are known to be weaker than smaller objects of the same material. Models of size-dependent strength¹⁵ imply that strength should scale as $R^{-3/(m+3)}$ where $m = 8.1$ for ice. Thus the models suggest that the strength of a KBO with $R = 100 \text{ km}$ should be $(10^7)^{-0.27} (= 0.01)$ times the laboratory strength of ice, that is, $\sim 2 \times 10^5 \text{ dyn cm}^{-2}$.

The second possibility that can explain the trend in Fig. 2 is that the KBOs in our sample are all about the same size. The difference in intrinsic brightness would then be due to differences in albedo, the intrinsically faint objects being composed of a darker material than the brighter objects. If the dark material has a greater material strength than the lighter material, the dark objects could support more overlying material and hence retain their irregular shapes to greater diameters than could the light-coloured KBOs (E. Asphaug, personal communication).

What mechanism is responsible for the production of irregularly shaped objects? One possibility is that the objects in our survey retained their original shapes from the time they stopped growing. An alternative possibility is that the irregular shapes are shards from collisions. Calculations^{16–18} have shown that objects with diameter $> 100 \text{ km}$ cannot be shattered by collisions in the present-day Kuiper belt; this is because collisions between such large targets and impactors that are large enough to disrupt these targets are too infrequent. If the KBOs have albedos similar to those of short-period comets, then their irregular shapes are the result of primordial formation processes. □

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Quantum-well states in copper thin films

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A standard exercise in elementary quantum mechanics is to describe the properties of an electron confined in a potential well. The solutions of Schrödinger's equation are electron standing waves—or 'quantum-well' states—characterized by the quantum number n , the number of half-wavelengths that span the well. Quantum-well states can be experimentally realized in a thin film, which confines the motion of the electrons in the direction normal to the film: for layered semiconductor quantum wells, the aforementioned quantization condition provides (with the inclusion of boundary phases) a good description of the quantum-well states. The presence of such states in layered metallic nanostructures is believed to underlie many intriguing phenomena, such as the oscillatory magnetic coupling of two ferromagnetic layers across a non-magnetic layer^{1,2} and giant magnetoresistance³. But our understanding of the properties of the quantum-well states in metallic structures is still limited. Here we report photoemission experiments that reveal the spatial variation of the quantum-well wavefunction within a thin copper film. Our results confirm an earlier proposal⁴ that the amplitude of electron waves confined in a metallic thin film is modulated by an envelope function (of longer wavelength), which plays a key role in determining the energetics of the quantum-well states.

The standard quantization condition on an electron of wavevector k within a potential well of width d is:

$$2kd + \phi = 2\pi n \quad (1)$$

This equation expresses the requirement that, as the electron 'bounces' back and forth within the well, the length of a round trip must (apart from end corrections embodied in the phase ϕ) equal an integral number of wavelengths. For fixed n , an increase of d results in a decrease of k . Because the conduction electron's

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energy usually increases with increasing k in noble metals (for example, in Cu, Ag and Au), equation (1) implies a decrease of the quantum-well (QW) energy with increasing d . However, previous photoemission experiments on metallic QW states show that the QW energies in fact increase with increasing film thickness^{4–6}. This apparent contradiction can be circumvented⁵ by rewriting equation (1) in terms of a new quantum number ν :

$$2(k_{\text{BZ}} - k)d - \phi = 2\pi\nu \quad (2)$$

where $k_{\text{BZ}} = \pi/a$ is the Brillouin-zone vector, a is the atomic spacing, $\nu = m - n$, and m is the number of atomic layers within the well. Equation (2) now gives an *increase* of the QW energy with increasing d , as observed experimentally. Is this merely a book-keeping trick, or is there some underlying physical significance? Noting that the electron wavevectors near the Fermi level in metals are close to the Brillouin-zone boundary ($k \approx k_{\text{BZ}}$), it has been proposed⁴ that electron waves under this situation should carry an envelope function with wavevector ($k_{\text{BZ}} - k$) that modulates the amplitude of the fast oscillating electron wave, as illustrated schematically in Fig. 1. Thus, equation (2) represents the quantization condition of the envelope function, with the quantum number ν simply being the number of nodes of the envelope function.

To investigate this idea experimentally, we developed a technique for mapping the spatial variation of the wavefunction in a QW. The method is analogous to sensing the standing-wave nodal structure of a vibrating string by lightly touching the string at different positions: touching the string at an antinode causes considerable damping of the vibration, but touching the string at a node has little effect. Thus by systematically changing the contact position along the string, it is possible to map out the spatial variation of the vibration amplitude for a given vibrational mode. For the case of the Cu QW state, the analogue of the 'touch' is provided by a thin Ni film (~ 1 monolayer, ML) inserted into the Cu QW. We chose Ni as the 'touch' layer for both structural and electronic reasons. Structurally, Ni shows pseudomorphic growth on Cu(100) so that it should not significantly affect the epitaxial growth of the Cu layer. Electronically, the band structure of Ni is sufficiently different from that of Cu that it offers an effective barrier to the free propagation of electrons between the Cu layers. By inserting the Ni layer into different positions of the Cu QW, it should therefore be possible to map out the spatial variation of the envelope function for a given QW state. Experimentally, the samples were prepared in ultrahigh vacuum ($\sim 10^{-10}$ torr). A 15 ML Co layer was first grown onto a Cu(100) single crystal to serve as the Co substrate. The Cu QW was then built by epitaxially growing the Cu film onto the Co substrate⁶. To vary systematically the Ni position within the Cu QW, we fabricated a double-wedge sample as illustrated in Fig. 2a: two identical Cu wedges were tapered in directions at right angles, with 1 ML Ni grown in between. Moving along the diagonal direction BD, the total Cu thickness is constant, but the Ni layer is swept continuously from one side of the Cu QW to the other to sense the expected envelope function. Moving along the other diagonal, AC,

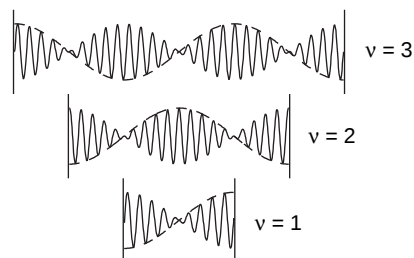


Figure 1 Schematic drawing of the QW wavefunctions at a given energy for $\nu = 1, 2$ and 3. The amplitude of the fast-oscillating electron wave is modulated by the envelope function (dashed line).

has the effect of varying the total Cu thickness while keeping the Ni barrier in the centre. We shall consider both BD and AC variations.

Photoemission spectroscopy is an ideal tool for the QW study because the photoemission intensity is proportional to the number of electrons at the given energy (the density of states). Thus, the QW states (which exist only at certain discrete energy levels) will manifest themselves as peaks in the photoelectron energy spectrum. Our photoemission experiments were performed at the Advanced Light Source, and take advantage of the high brightness of this third-generation synchrotron radiation facility. A high photon flux ($> 10^{12}$ photons s^{-1}) can be delivered into a small spot size (50–100 μm) on the sample. Combining this with the use of wedge-shaped samples (Fig. 2a), it is possible to investigate a range of layer thicknesses by moving the wedge sample laterally through the illuminated spot. Figure 2b shows the photoemission intensity at the Fermi level across the sample. Two types of oscillations are visible. Scanning vertically (that is, parallel to AC), we see oscillations having a period of 5.6 ML. This oscillation has been seen before^{4,6}, and the intensity peaks correspond to the QW states with different index ν at the Fermi level within the Cu film. Scanning horizontally (that is, parallel to BD), we see oscillations that have not been seen before: each QW state labelled by index ν now oscillates as the Ni layer is swept through the Cu film. Qualitatively, these oscillations reflect the spatial variation of the envelope function of the Cu QW states as sensed by the Ni layer. For further understanding it is necessary to consider the wavefunction matching conditions at the boundaries embodied in the phase ϕ . It turns out in our case that ϕ is small, which means that the envelope function is 'trying' to place its antinodes at the boundaries⁴. In the

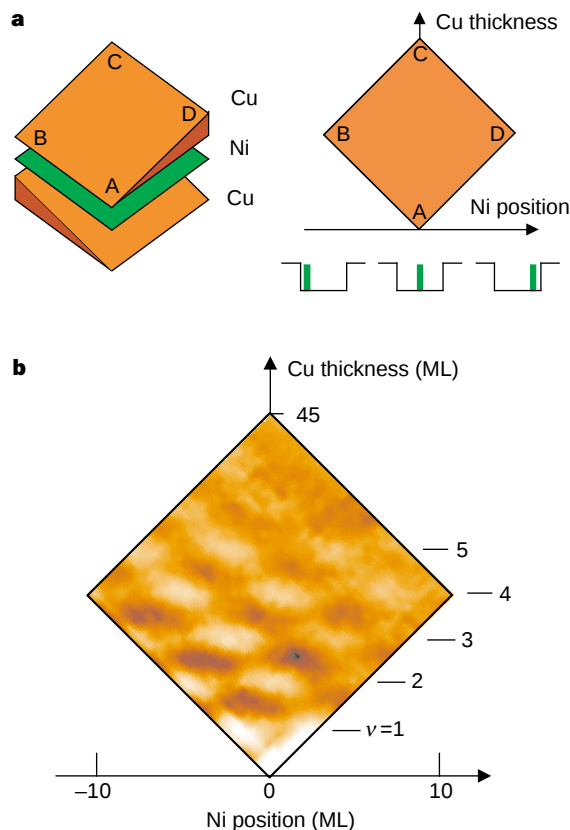


Figure 2 Spatial variation of the QW wavefunctions mapped by photoemission. **a**, Schematic drawing of the double-wedge sample that allows independent variation of the Ni layer position and the total Cu thickness. **b**, The two-dimensional image of the normal-emission photoemission intensity at the Fermi level taken over the double-wedge. The intensity variation with the Ni position reflects the QW envelope function as shown in Fig. 1.

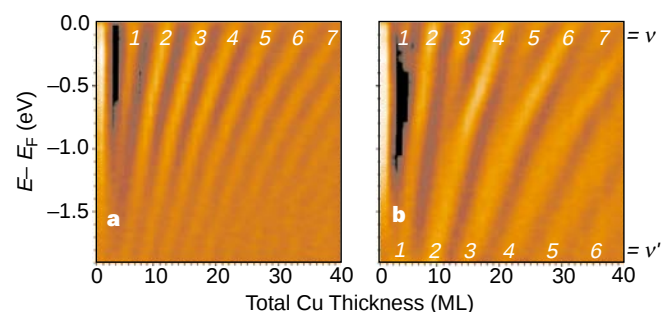


Figure 3 Images of the photoemission energy spectra versus the total Cu thickness for the symmetrical double QW Cu/Ni/Cu. **a**, 0 ML Ni; **b**, 1 ML Ni. With the presence of the Ni, each degenerate QW state at low energy splits into two states at high energy with even and odd parities.

presence of the Ni barrier, the QW states will be least perturbed when the barrier is itself near an antinode of the envelope function. When the barrier is close to a node of the envelope function, there will be difficulty in satisfying the wavefunction matching condition, leading to a damped or suppressed QW state. Thus the maximum and minimum intensities in the scans parallel to BD correspond respectively to the antinodes and nodes of the envelope function, and we associate the $\nu = 1, 2, 3$ states in Fig. 2b with the schematic wavefunctions of Fig. 1.

When the Ni barrier is at the centre of the Cu (along line AC in Fig. 2a), it separates the Cu layer into a symmetric double QW system. This spatial symmetry requires that the envelope function is either symmetric (even parity with an antinode at the centre) or antisymmetric (odd parity with a node at the centre) relative to the centre of the well. The parity of the envelope function has the observable consequence, as can be seen along the AC line in Fig. 2b, that QW states of even ν are more intense than those of odd ν . We now examine this in more detail at different electron energies. Noting that the Ni serves as a potential energy barrier, the propagation of the Cu electron wave across the Ni layer should become more difficult at lower energies. This is associated with a bandgap of bulk Ni (at ~ 1 eV below the Fermi level) within which free propagation of electrons is prohibited⁷. Thus, we expect the following two limiting cases based on elementary quantum mechanics. At low energies, the Ni barrier will completely decouple the two Cu QWs to result in degenerate QW states in the two Cu wells. At high energies, the degeneracy of the QW states in the two Cu wells will be lifted by the propagation of the Cu electrons across the Ni barrier, resulting in two non-degenerate QW states with odd and even parities. Figure 3b shows the evolution of the QW states at different energies with 1 ML Ni at the centre of the Cu well (Fig. 3a shows the case without the Ni barrier for comparison). At low energies ($E - E_F < -1$ eV, where E_F is the Fermi energy), we see the degenerate QW states of the separated Cu wells labelled by the quantum number ν' . As the energy increases, we see these states split into pairs. As compared with Fig. 3a, which corresponds to the case with no Ni barrier, the degenerate states labelled by ν' evolve into the $\nu = 2\nu' - 1$ and $\nu = 2\nu'$ states of the Cu well of twice the thickness. Figure 3b therefore demonstrates that the coupling of the two Cu QWs results in pairs of QW states with odd and even parities.

The qualitative analysis presented here needs to be tested with quantitative theoretical calculation. For example, tight-binding modelling⁵ might provide useful insight into the orbital character of the QW wavefunctions. The aim is to reach a level of theoretical understanding and experimental control that will enable us to consider 'wavefunction engineering' of metallic layered systems, comparable to the 'bandgap engineering' that is now well established in semiconductor layered structures. □

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Enhancement of surface self-diffusion of platinum atoms by adsorbed hydrogen

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Surface diffusion of atoms is an important phenomenon in areas of materials processing such as thin-film growth and sintering. Self-diffusion (that is, diffusion of the atoms of which the surface is comprised) has been much studied on clean metal and semiconductor surfaces^{1,2}. But in most cases of practical interest the diffusion happens on surfaces partly covered by atoms and molecules adsorbed from the gas phase. Adsorbed hydrogen atoms are known to be capable of both promoting and inhibiting self-diffusion^{3–7}, offering the prospect of using adsorbed gases to control growth or sintering processes^{8–11}. Here we derive mechanistic insights into this effect from observations, using the scanning tunnelling microscope, of hydrogen-promoted self-diffusion of platinum on the Pt(110) surface. We see an activated Pt–H complex which has a diffusivity enhanced by a factor of 500 at room temperature, relative to the other Pt adatoms. Our density-functional calculations indicate that the Pt–H complex consists of a hydrogen atom trapped on top of a platinum atom, and that the bound hydrogen atom decreases the diffusion barrier.

We have studied the influence of gas adsorbates on the diffusion of metal adatoms on the reconstructed Pt(110)-(1 × 2) surface, for which every second close-packed Pt row is missing (Fig. 1). This is an ideal model system because the motion of Pt adatoms is one-dimensional and is restricted to the troughs between close-packed rows of Pt atoms.

The experiments were performed in a standard ultrahigh vacuum system equipped with a fast-scanning, variable-temperature scanning tunnelling microscope (STM) that was built in our laboratory at the University of Aarhus. Platinum adatoms were deposited onto the clean (1 × 2) reconstructed surface by heating a 99.995% pure Pt wire. Hydrogen was coadsorbed from a constant background pressure.

To gain detailed insight into the migration of the Pt adatoms, we have acquired a large number of consecutive STM images

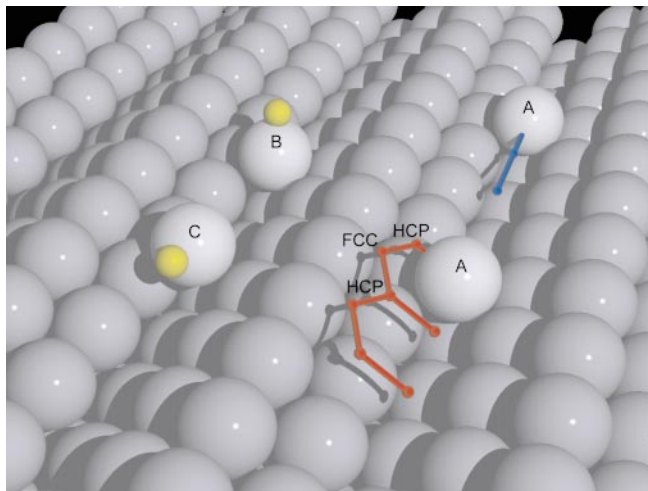


Figure 1 Illustration of the Pt(110)-(1 × 2) surface, consisting of one-dimensional troughs separated by ridges of close-packed Pt rows. Pt adatoms are restricted to move along the troughs. Two single adatoms in their equilibrium positions are shown (A) and the two possible diffusion paths, direct (blue) and indirect over

face-centred cubic (f.c.c.) and hexagonal close packed (h.c.p.) sites on the ridge (red), are indicated. In each case, two consecutive hops are shown. The Pt-H complex is also shown in the equilibrium state (B) as well as in the transition state for diffusion (C) found in the density-functional theory calculations.

(256 × 256 pixels) of the same area on the surface. The acquisition time for the individual sequential STM images was adjusted to suit the mobility of the diffusing Pt adatoms, and ranges from 1 to 20 s. When the STM images are played back as an STM 'movie', a direct visualization of the diffusion process is obtained. Typically, an STM movie consists of up to several thousand images, each showing 10 to 100 Pt adatoms.

Our main finding is that we have directly imaged, with the STM, the formation of intermediate Pt-H complexes, which have a significantly larger diffusivity than the other Pt adatoms. These Pt-H complexes show up in the STM movies as brighter Pt adatoms (increase in apparent height $\approx 0.4 \text{ \AA}$). After some time, a bright adatom reverts to a normal brightness. One such event is presented in the STM images in Fig. 2, which are taken from an STM movie recorded at 303 K and at a hydrogen pressure of 7×10^{-7} mbar. In the first image, all Pt adatoms look and behave the same. In the following four images, one single adatom appears much brighter and moves several lattice spacings from image to image. In the same period, only one of the other Pt adatoms performs a hop to a neighbouring lattice site.

Thermal desorption studies¹² have shown that H desorbs around room temperature, and thus the hydrogen coverage is determined by an equilibrium between adsorption and desorption. We estimate the hydrogen coverage at 300 K and a pressure of 7×10^{-7} mbar to be ~ 0.5 H atoms per (1 × 2) unit cell. Experimentally, we find that the diffusivity of the Pt adatoms on Pt(110)-(1 × 2) is constant with time for a given hydrogen coverage.

As shown in Fig. 3, we find that at the lowest background pressure of $\sim 7 \times 10^{-11}$ mbar (mainly H₂) the Pt adatoms at 303 K have a mean-square displacement, $\langle x^2 \rangle$, of ~ 0.002 nearest-neighbour spacings² per second, consistent with our previous finding¹³ of a Pt self-diffusion activation energy of 0.81 eV. For this and the following comparisons, we use the prefactor $10^{10.7} \text{ s}^{-1}$ (ref. 13). No bright Pt adatoms are observed in this pressure range. Increasing the hydrogen pressure to 4×10^{-8} mbar and further to 7×10^{-7} mbar, the Pt adatoms are found to diffuse faster, and bright adatoms appear. At the highest hydrogen pressure, the observed value of $\langle x^2 \rangle$ for the bright adatoms is on average ~ 500 times larger than without hydrogen, corresponding to a reduction of the diffusion barrier by 0.16 ± 0.02 eV.

The lifetime of the bright adatoms at 303 K and 7×10^{-7} mbar follows an exponential decay with a mean lifetime of ~ 18 s, corresponding to an energy barrier of ~ 0.8 eV. As the images in

the movies are taken with time intervals which are significant relative to the mean lifetime, the observed increase in diffusivity of the other adatoms is probably due to the formation of short-lived bright adatoms (Pt-H complexes), some of them thus not registered.

We have interpreted these STM observations in the light of density-functional calculations of the effect of H atoms on the diffusion of Pt adatoms. In our calculations, we use a nine-layer slab of Pt(110)-(1 × 2) with Pt and H adsorbed in a (2 × 2) unit cell. The top three Pt layers and the adsorbate degrees of freedom are fully relaxed for each configuration studied. Ionic cores are described by ultra-soft pseudopotentials¹⁴, and the Kohn-Sham one-electron valence states are expanded in plane waves with kinetic energies up to 25 Ry (ref. 15). The surface Brillouin zone is sampled at eight special **k**-points. Exchange-correlation effects are described by the generalized gradient approximation (PW91)¹⁶.

Our calculations show that the most stable configuration of an adsorbed H atom is on top of a Pt adatom (configuration B in Fig. 1). The adsorption energy is 1.07 eV per H₂ molecule, essentially identical to the calculated adsorption energy in the most stable bridge site on the Pt(110)-(1 × 2) surface with no Pt adatoms. From thermal desorption experiments, an adsorption energy of 0.9 eV per H₂ molecule has been deduced¹². In the calculations, hydrogen atoms are found to bind more weakly (by ~ 0.2 eV) to other sites.

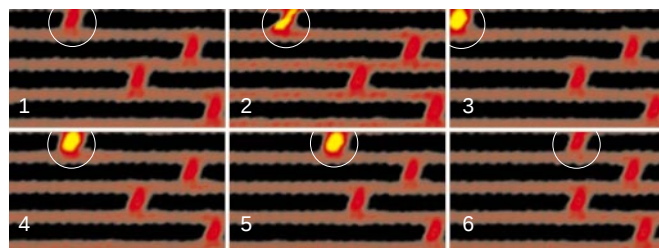


Figure 2 Six STM images ($56 \times 31 \text{ \AA}^2$) extracted from a typical STM movie. Shown is the development of a normal Pt adatom into a bright Pt-H intermediate complex, and then back to a normal Pt adatom again. All STM images were obtained in the constant-current mode with tunnel resistances above 100 MΩ in which case the influence of the tip was found to be negligible. The Pt adatom of interest is marked by a white circle. (Examples of STM movies are available at <http://www.ifa.au.dk/camp/hptmovies>).

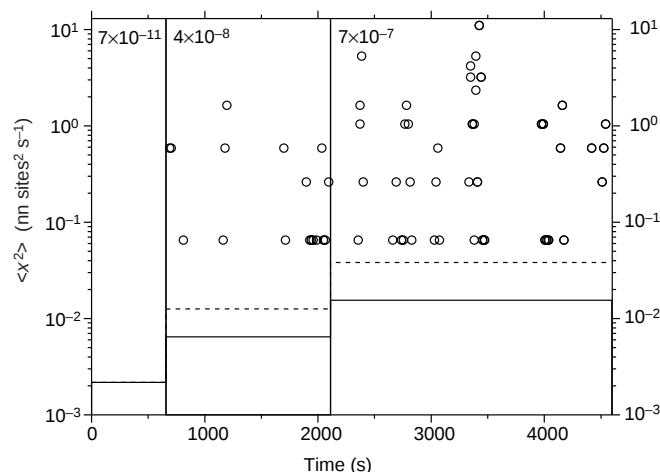


Figure 3 Mean-square displacements ($\langle \chi^2 \rangle$) (in nearest-neighbour (nn) sites² s⁻¹) for different H background pressures. Images were taken at a sample temperature of 303 K and with 15 s per image. Vertical lines indicate times when the H background pressure was increased; first, to 4×10^{-8} mbar and second, to 7×10^{-7} mbar. Open circles, the $\langle \chi^2 \rangle$ for image-to-image displacements of individual Pt-H complexes; dashed lines, the average $\langle \chi^2 \rangle$ of all Pt adatoms; full lines, the average $\langle \chi^2 \rangle$ of the other Pt adatoms in this pressure interval.

We can estimate how a Pt adatom will change appearance in the STM when a H atom is adsorbed on top of it by calculating the local density of states (LDOS) at the Fermi level (ref. 17). The H atoms induce an increase in this LDOS. The effect is very similar to the 'brightness' effect observed experimentally (Fig. 2), strongly suggesting that the bright Pt adatoms are associated with Pt-H complexes with top-bound H atoms.

A Pt adatom on the clean Pt(110)-(1 × 2) surface is found to have two possible diffusion paths: a direct path along the troughs, and an indirect path where it moves to the ridges on the (111) micro-facets of the Pt(110)-(1 × 2) surface (Figs 1 and 4). We determine the energy and configuration of the transition state by varying a single adatom coordinate along the diffusion path, and minimizing the energy with respect to all the other degrees of freedom. The transition state is the maximum energy along this path. We find that the barrier for Pt diffusion is 0.94 eV, slightly larger than found experimentally¹³. We associate this difference with the approximate treatment of exchange-correlation effects and with the relatively small unit cell, which must be used owing to the large computational demands of the problem.

The barriers for the two different diffusion paths are affected differently when a hydrogen atom is associated with the Pt adatom. The indirect path along the ridge has an increased barrier, while the direct path has a barrier which is lowered by 0.09 eV (Fig. 4), in reasonable agreement with the value found experimentally (0.16 eV). We therefore propose that the effect of H atoms on the diffusivity of Pt adatoms on Pt(110)-(1 × 2) is to bind on top of the Pt adatoms, and reduce significantly the barrier of the direct diffusion path.

The calculations also show that no general rule exists to predict whether an adsorbate will promote or inhibit the diffusivity: the effect seems to depend on the local geometry of the diffusion path. Furthermore, the H atom changes place during the diffusion event. At the transition state it slides down the side of the Pt adatom (C in Fig. 1). If we do not allow for this extra relaxation, no lowering of the diffusion barrier occurs.

A question that remains is why only ~1% of the Pt adatoms are observed to be in the bright state. We suggest that trace amounts of CO compete with H atoms for the Pt adatom sites¹⁸; our calculations show that CO strongly prefers these adatom sites. We have tested this hypothesis experimentally by increasing the CO back-

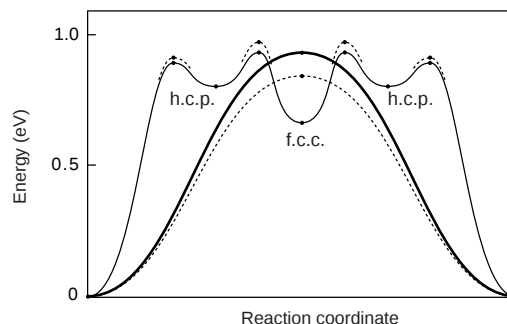


Figure 4 The calculated energy along the two different diffusion paths shown in Fig. 1. The thick (thin) curves indicate the direct (indirect) path. The diffusion paths on the clean Pt surface are shown by the solid curves; the dashed curves show the effect of forming the Pt-H complex. The equilibrium structure and the structure at the transition state for the direct process (the lowest barrier) are shown respectively as B and C in Fig. 1.

ground pressure to 5×10^{-10} mbar, and as expected this suppresses the formation of the intermediate Pt-H complexes: a detailed discussion of these tests will be published elsewhere. Our calculations show that the finite lifetime of the Pt-H complexes is related to the diffusion of a CO molecule from the Pt ridge to the Pt adatom site (barrier ~0.8 eV), thus replacing the H atom.

The present finding of a mechanism for adsorbate-promoted diffusion has implications for many processes in which gas-surface interactions are important (such as sintering, chemical vapour deposition, catalysis, and crystal growth). Adsorbate-modified diffusion explains why sintering phenomena depend crucially on gas composition, and why adsorbates can act as surfactants in metal-on-metal growth. With a better systematic understanding of the phenomenon, gas adsorbates could be used to control diffusion of adatoms in surface processes. Hydrogen is a very good candidate for such an adsorbate, as it diffuses fast and, for most materials, can be removed from the surface by mild heating. □

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Non-centrosymmetric superlattices in block copolymer blends

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Materials with a macroscopic electric polarization display a variety of useful properties, such as piezo- and pyroelectricity and second-order nonlinear optical activity¹. Macroscopic polarization results when dipolar molecules are orientated in the same direction, or when ions are organized in a non-centrosymmetric crystal structure². Centrosymmetric molecules have no dipole moment and so cannot generate a macroscopic polarization. Non-centrosymmetry in amorphous materials can be engineered by depositing particular sequences of layers on top of each other, or by applying external fields (generally electric) to orientate the molecules³. Here we report the formation of a non-centrosymmetric structure in an amorphous material through spontaneous self-assembly. Block copolymers are known to form ordered structures at the microscale owing to segregation of the different blocks^{4,5}. We show that a mixture of a ternary triblock copolymer and a binary diblock copolymer will organize itself into a non-centrosymmetric layered structure in which the layers are occupied by different blocks. The structure is periodic with a length scale of around 60 nm.

The formation of liquid-crystalline non-centrosymmetric materials from molecules which are themselves non-centrosymmetric but not chiral has been much debated in the past two decades^{6,7}. In particular, molecules consisting of three or four sub-groups, α - β - γ and α - β - γ - α have been suggested as good candidates to obtain longitudinal ferroelectric smectics⁸⁻¹⁰ (that is, materials where non-centrosymmetric molecules self-assemble into stacks of liquid-like layers with all the molecules being orientated in the same direction). Such materials have been synthesized and studied^{11,12}. Achiral banana-shaped molecules forming antiferroelectric chiral smectics have been reported, which can be transformed into ferroelectric materials by application of an electric field^{13,14}. In the case of low-molecular-weight self-assembling compounds, smectic periodicity lies in the range of a few nanometres. To achieve smectic order with larger layer thickness (and thus larger periodicity) through self-assembly, it is natural to use linear block copolymers: these molecules consist of long sequences of chemically different monomers linked together by a covalent bond. Thanks to the improvement of synthetic techniques, it is now possible to make A-B-C triblock copolymers with well controlled molecular architecture, that is, with well controlled block lengths. Depending on the chemical structure and the length of blocks, these molecules form very interesting mesophases¹⁵⁻²⁰. In most cases, centrosymmetric structures have been observed. The only exception reported so far was a helical morphology, where the occurrence of right- and left-handed helices suppressed the formation of a well developed long-range-ordered structure²¹. One of us (R.S.) has proposed that by mixing suitably chosen A-B-C triblock copolymers with A-C diblock copolymers it should be possible to obtain self-assembled non-centrosymmetric periodic lamellar structures orientated on the micrometre scale.

In linear A-B-C triblock copolymers, the simplest lamellar (smectic) mesophases consist of well segregated layers of different monomers A, B and C. Indeed, chemically different polymer species are, in general, incompatible, and segregation effects are strong. Thus, the energetically favoured lamellar arrangement is (ABCCBA)_n, which is centrosymmetric. Compared to a non-centrosymmetric sequence (ABCABC)_n, high-energy AC interfaces have been replaced by low-energy AA and CC contacts. The situation is much less trivial for lamellar structures of mixtures of triblock and diblock copolymers. Even if we minimize the number of interfaces between different chemical species, we are still left with four different types of possible arrangements (Fig. 1). But even though all the situations shown in Fig. 1 have the same number of high-energy interfaces between incompatible A and C layers as well as between A and B layers, they differ in some respects. In particular, they differ in the number and order of athermal interfaces between the A-layers formed by A-monomers pertaining to triblock chains and those of diblock ones (which we denote as A- and a-layers and monomers, respectively). In fact, the copolymer chains assembled into layers are stretched in order to minimize the interface and thus the number of contacts between unlike species. Generally the stretchings in A- and a-layers are different, as the stretching in triblocks is governed by A-B and B-C interactions rather than A-C interaction as in diblocks. So, for Aa contact, the interdigitation profile is asymmetric: the strongly stretched chains invade more readily the region occupied by the less-stretched counterpart chains. As a consequence, the system gains an (exchange) energy f_a by replacing an AA contact between triblock layers and an aa contact between diblock layers by two contacts Aa between a triblock and a diblock layer. Similar considerations apply to C and c blocks. Therefore, depending on the exchange energies f_a and f_c , different situations can occur.

Calculations show that the exchange free energy of the system favours the desired non-centrosymmetric arrangements ($f_a, f_c < 0$, Fig. 1d) if the asymmetry is strong enough for both aA and cC

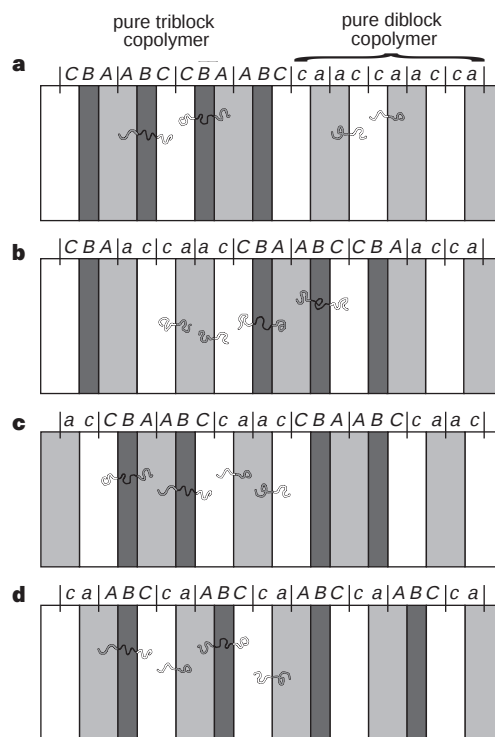


Figure 1 Diagram showing all possible lamellar morphologies of blends of ABC and ac block copolymers. **a**, Macrophase separation. **b**, Random sequence. **c**, Centrosymmetric sequence. **d**, Non-centrosymmetric sequence.

†Deceased.

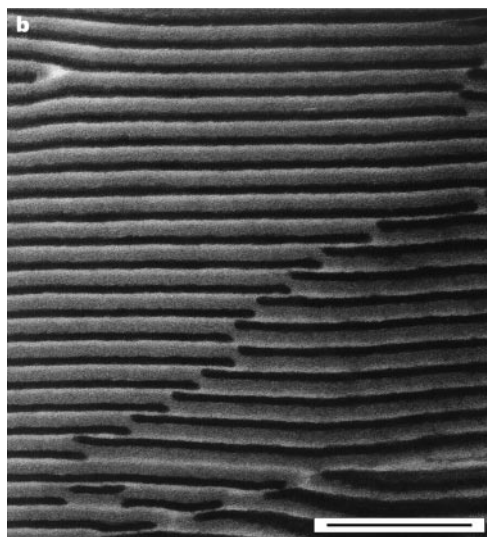


Figure 2 Transmission electron micrographs of various blend compositions. The specimens were stained with OsO_4 . **a**, Transition from excess of pure triblock (top) to non-centrosymmetric blend structure (bottom) in a blend of 75 mol.% SBT

and 25 mol.% **b**, Non-centrosymmetric blend structure in a blend of 50 mol.% SBT and 50 mol.% st. Scale bar in **a** and **b**, 250 nm.

contacts. Two different centrosymmetric arrangements of double layers will be favoured for $f_a > 0, f_c < 0$ (Fig. 1c), and $f_a < 0, f_c > 0$ (not shown), respectively. A random sequence of diblock and triblock copolymers is favoured in the case of $f_a, f_c = 0$ (Fig. 1b), and macroscopically demixed domains of diblock and triblock copolymers are favoured for $f_a, f_c > 0$ (Fig. 1a).

Which morphology the mixture will in fact exhibit depends on a subtle balance of the polymer entropic conformational effects (described above) and the long-range interactions between the layers. The latter (van der Waals and polar interactions) depend on layer thickness and have been extensively discussed in the literature in the context of the possible existence of longitudinal ferroelectric smectics. For thick layers (long chains), the exchange free energy can dominate van der Waals interactions and govern the blend structuring. Thus, the observed morphologies could also provide us (after accounting for long-range interactions) with important (even though indirect) information concerning such an interdigitation of chain ends belonging to opposite layers.

Blends of the two block copolymers given in Table 1, polystyrene-block-polybutadiene-block-poly(*tert*-butyl methacrylate) triblock copolymer SBT and polystyrene-block-poly(*tert*-butyl methacrylate) diblock copolymer st, with various molar ratios between SBT and st, have been investigated by transmission electron microscopy (TEM) and small angle X-ray scattering (SAXS). Both pure block copolymers form centrosymmetric lamellar morphologies (stts and SBTtBS). The SAXS pattern of the pure SBT indicates a long period of 88 nm, whereas the blend of 50 mol.% of each shows a distance of 67 nm. Both are in good agreement with the dimensions obtained from TEM. (We are taking into account the fact that the lateral dimensions of poly(*tert*-butyl methacrylate) shrink by a factor of 3 in the electron beam of the TEM.) The pure st does not give enough scattering contrast to be investigated with this method. In the TEM, staining with OsO_4 lets the polybutadiene phase (B) appear black, whereas the polystyrene phase (S) is only slightly darkened to a light grey. Poly(*tert*-butyl methacrylate) (T) does not react with OsO_4 at

all, and remains white. The T lamellae appear thinner than those of S due electron-beam damage of the T in the TEM.

In Fig. 2a we show a blend with an excess of SBT. A macrophase separation between the pure triblock copolymer and a superlattice containing both triblock and diblock copolymer is observed. Whereas the pure SBT-phase shows centrosymmetry, the superlattice is non-centrosymmetric. The distances between the black B lamellae of the blend structure are no longer alternating like in the pure SBT. The interface between the blend SBTs and pure excess triblock SBTtBS is remarkable. Every second triblock lamella continues, and the other is replaced by a diblock layer that has the same outer blocks S and T and thus fits into the pattern. This transition from one domain into the other, which is also associated with a transition of symmetry, proves the superlattice sequence of alternating di- and triblock lamellae.

Figure 2b shows a lamellar morphology with long-range order, found for a blend of equimolar composition. All three lamellar phases are clearly visible in the sequence 'black-grey-white-black' which corresponds to the sequence B-S-T-B. In contrast to the pure SBT, there is an interface between S and T (the borderline between white and grey layers) which belongs to the internal surface of the diblock copolymer st. This leads to an alternating sequence of triblock and diblock copolymer chains SBTtsSBTtsSBTts, which is a non-centrosymmetric superlattice with asymmetric layers sS and tT. These layers are composed of diblock and triblock chains coming from opposite sides. According to the TEM analysis, almost the whole sample consists of the SBTts superlattice structure.

Due to this asymmetry, both orientations of the sequence SBTts and its reverse counterpart TBSst are not compatible. As a consequence, oppositely orientated stacks of lamellae do not fit together, and have a grain boundary where the B-lamellae of the triblock are interrupted. In the pure diblock and triblock copolymer morphologies, such a defect is not possible, and so has not been found in the TEM images. At the boundary parallel to the lamellae, there is a double layer of one block copolymer inverting the sequence. This typical defect may be seen in the lower part of Fig. 2b, where the sequence 'grey-white-grey' indicates a double layer of diblocks 'stts'. The situation is shown schematically in Fig. 3. The appearance of both orientations within one view proves that this defect is not an artefact of sectioning or projection, but clearly indicates the non-centrosymmetry of the blend structure. We also observed the formation of the lamellar periodic non-centrosymmetric superlattice in a blend of polybutadiene-block-polystyrene-block-

Table 1 Polymer properties

Polymer	M_n (kg mol ⁻¹)	M_w/M_n	w_{PS}	w_{PB}	w_{PT}	ϕ_{PS}	ϕ_{PB}	ϕ_{PT}
SBT	160	1.04	0.34	0.33	0.34	0.32	0.34	0.33
st	98	1.03	0.50	-	0.50	0.49	-	0.51

M_w and M_n are respectively the weight and number averages of the molecular mass; w_x is the weight fraction of x; ϕ_x is the volume fraction of x. PS, polystyrene; PB, polybutadiene; PT, poly(*tert*-butyl methacrylate).

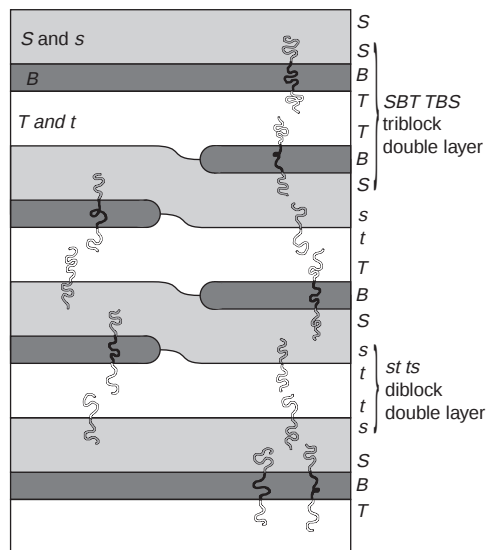


Figure 3 Chain orientations at a morphological defect of a lamellar non-centrosymmetric blend of SBT and st block copolymers.

poly(methyl methacrylate) and polybutadiene-block-poly(methyl methacrylate) (not shown).

The results that we report here have shown that non-centrosymmetric materials may be produced by amorphous self-assembling block copolymer blends. By changing the molecular weights of the blend components, control of the periodic length should be possible: this concept should make accessible periodic non-centrosymmetric materials with a well defined long-range periodicity that is within the mesoscopic length scale. Other monomers might be considered for introducing useful molecular properties into such a non-centrosymmetric material. □

Methods

Synthesis. Block copolymers were synthesized by sequential anionic polymerization of different monomers. A polystyrene-block-polybutadiene-block-poly(*tert*-butyl methacrylate) triblock copolymer (SBT) and a polystyrene-block-poly(*tert*-butyl methacrylate) diblock copolymer (st) were synthesized in tetrahydrofuran in the presence of lithium alkoxides using *sec*-butyllithium as initiator. Details are given in ref. 22; TBMA is treated as MMA except for the polymerization temperature, which is increased over 2 h from -60°C to -40°C . The characteristics of the block copolymers are given in Table 1.

Sample preparation. The blend components were dissolved together in chloroform which was evaporated over several weeks. For further equilibration, the dry films were annealed at 150°C for 6 h in vacuum.

Transmission electron microscopy. This was performed using a ZEISS 902 transmission electron microscope operating at 80 kV in the bright-field mode. Ultrathin sections of the samples were obtained using a Reichert ultramicrotome equipped with a diamond knife.

Small angle X-ray scattering. This was performed using a Bruker AXS 2D-SAXS Nanostar instrument, operating with Cu $K\alpha$ radiation, at the Application Laboratory of A. Paar KG in Graz, Austria.

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Unblocking of the Nares Strait by Greenland and Ellesmere ice-sheet retreat 10,000 years ago

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The extent of glaciation at the northern margin of the Canadian/Greenland high-latitude Arctic region over the past 30,000 years is uncertain. Geological arguments have been made for Greenland and Ellesmere Island ice sheets that coalesced to block the Nares Strait¹, and for restricted ice sheets on the two islands² leaving the strait open, as it is today³. Distinguishing between these two possibilities would provide significant constraints on present understanding of the past circulation between the Arctic and Atlantic oceans^{4,5}, on estimates of past ice-volume⁶, and on the response of the Greenland ice sheet to climate change⁷. Radio-carbon analyses provide dates for the deglaciation of the islands' coasts, but do not yield information on whether ice filled the strait. Here we present measurements of cosmogenic ³⁶Cl that has accumulated *in situ* in erratics and glacially polished bedrock on islands within the Nares Strait. These data allow us to determine the time for which the rocks have been recently exposed to the atmosphere, and thus the age of the final deglaciation of the strait. We show that Greenland and Ellesmere ice sheets retreated from the Nares Strait about 10,000 years ago. The strait was filled with ice during the last glaciation, blocking this connection between the Arctic and Atlantic oceans, and supporting the model of extensive and long-lasting ice on land and sea in this region^{8–11}.

The Nares Strait, a body of water 500 km long and 20–100 km wide between Greenland and Ellesmere Island (Fig. 1), has no permanent ice cover and together with other arctic channels carries at present an estimated 30% of the total flow from the Arctic Ocean to the Atlantic Ocean¹². But in the past, expanded ice sheets filled the strait and drained northward to the Arctic Ocean and southward to Baffin Bay¹³. In the north, Greenland erratics and striated bedrock on Ellesmere¹⁴ and Hans islands¹⁵ record the extent, and northward movement, of the Greenland ice sheet. In the south, erratics and abraded bedrock on both sides of Smith Sound, including Pim Island, indicate southward flow of the coalescent Greenland and Ellesmere ice^{10,16,17}. Although the field evidence for the occupation of the Nares Strait by glaciers is unequivocal, the time when it was last glaciated has remained uncertain. If the Nares Strait was last glaciated before Late Wisconsinan times, then a scenario of Late Wisconsinan individual ice caps (the Franklin ice complex)² separated by a full-glacial sea³ would be supported. In this scenario, the northern margin of the Laurentide ice sheet is restricted, and the northwestern margin of the Greenland ice sheet is close to its present position. However, a Late Wisconsinan age for the glacial features would support the existence of the Innuitian ice sheet¹ that coalesced with the Laurentide ice sheet in the south and with the Greenland ice sheet in the northeast. These two scenarios provide entirely different palaeoclimatic interpretations of the ocean circulation during full-glacial times, the response of the Greenland ice sheet to full-glacial climate⁷, the flow patterns and thickness of past ice sheets^{6,7,18}, and the nature of the full-glacial climate in the high-latitude Arctic.

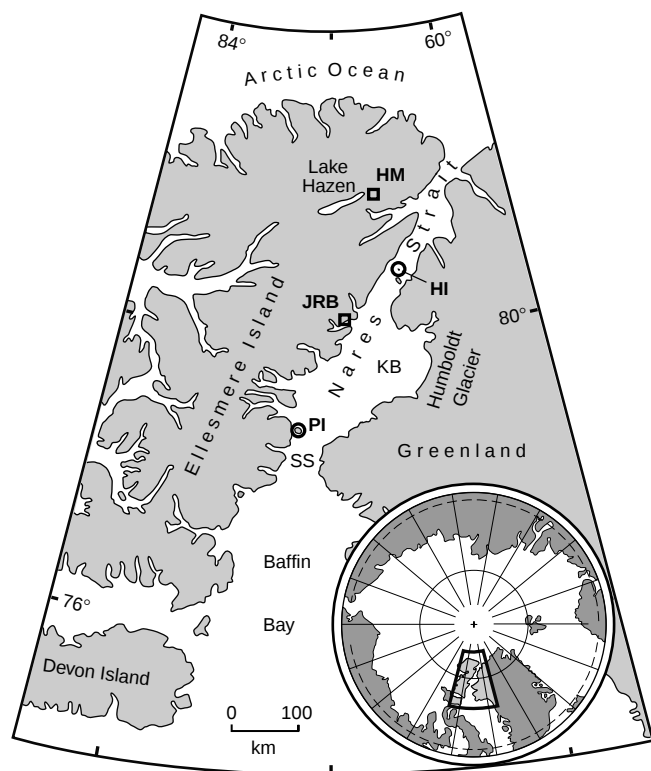


Figure 1 Location of samples for cosmogenic ^{36}Cl dating of late-glacial landforms in the Nares Strait area. Squares, sites used for comparison of ^{36}Cl and ^{14}C ages; circles, sites used for dating of deglaciation of the Nares Strait. Abbreviations of geographical names used in text: HM, Hazen moraines; JRB, John Richardson Bay; HI, Hans Island; PI, Pim Island; KB, Kane basin; SS, Smith Sound. Glacial striations on islands within Nares Strait indicate that ice flow was parallel to the strait, northward on Hans Island and southward on Pim Island. These directions of flow imply a significant thickness of combined Greenland and Ellesmere Island ice, with an ice divide over Kane basin.

We used the cosmogenic ^{36}Cl method¹⁹ to determine surface exposure ages of erratics and bedrock on Hans and Pim islands within the Nares Strait. If the Nares Strait was ice-free since long before the Last Glacial Maximum, bedrock and erratics on islands in the strait would yield cosmogenic exposure ages $>35,000$ yr. Conversely, if Nares Strait was occupied by ice during the Last Glacial Maximum, cosmogenic ^{36}Cl ages would be $<15,000$ yr.

To demonstrate the applicability of the cosmogenic ^{36}Cl method in the Arctic and the validity of the production rates determined by our group elsewhere²⁰, we measured ^{36}Cl in rocks from early Holocene landforms: the Hazen moraines on Hazen plateau and an ice-contact delta in John Richardson Bay (Fig. 1 and Table 1). The moraines are well preserved, with sharp crests, steep slopes and numerous boulders. They extend to raised marine deposits dating between 8,130 and 6,995 ^{14}C yr (refs 3, 21), equivalent to 9,100–7,800 calendar years (cal. yr; correction after Taylor *et al.*²²). In John Richardson Bay, limestone bedrock littered with abundant boulders was exposed by the retreating ice margin. Marine shells associated with the adjacent ice-contact delta gave a ^{14}C age of 8,050 yr (ref. 23), or $\sim 9,000$ cal. yr. Average cosmogenic ^{36}Cl ages for the two features (Table 1 and Fig. 2) agree at the 1σ level with the ^{14}C age estimates. These results show that the ^{36}Cl method yields accurate ages in this region. They also indicate that if individual sample ages show low variability (that is, there are no obvious outliers), the best age estimate is the sample average.

To determine when ice sheets last occupied the Nares Strait, we collected samples of erratics and glacially polished bedrock from two islands in the strait (Fig. 1 and Table 1). On Hans Island, glacially sculpted, striated and plucked limestone bedrock indicates that ice flowed northward¹⁵ from the inferred ice divide in Kane basin¹³. Granite erratics on the bedrock were transported to this site from the interior of Greenland by the precursor of Humboldt Glacier (Fig. 1). On Pim Island, ubiquitous striations and stream-lined bedforms were formed on red granitic bedrock by the southward-flowing Smith Sound ice stream¹⁶. This ice deposited a veneer of till that includes limestone and sandstone erratics from the Proterozoic and Palaeozoic terranes to the north, as well as local granitic boulders.

All but two ^{36}Cl surface exposure ages postdate the Last Glacial Maximum (Table 1 and Fig. 2). On Hans Island, the mean age is

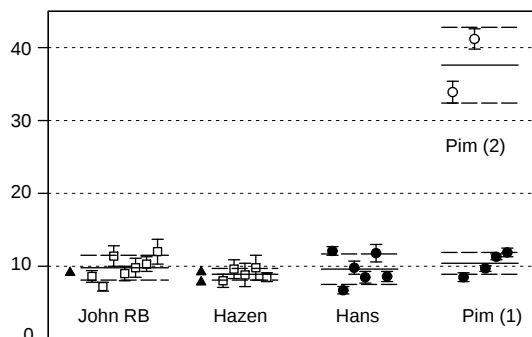


Figure 2 Cosmogenic ^{36}Cl exposure ages of samples from four locations in the Nares Strait area. Black triangles, ^{14}C ages (in calendar years) that we used to validate the ^{36}Cl method. Thick horizontal lines, average surface ages calculated assuming zero erosion; dashed lines above and below represent one standard deviation. Two Pim Island samples have exposure ages that differ from the others by more than five standard deviations. Because these older ages are probably due to ^{36}Cl inherited from previous exposure episodes, we removed them from further consideration. The remaining cosmogenic ^{36}Cl ages from Hans and Pim islands agree numerically with ^{14}C ages of about 9,000–10,000 calendar years for late glacial deposits from both coasts of the Nares Strait.

9,600 $\pm$ 2,100 yr and the maximum is 12,100 yr. Individual sample ages have a distribution similar to that for the test samples from John Richardson Bay. There is no systematic difference between lithologies, or between erratics and bedrock, and there is little chance of soil erosion (boulder uncovering) that could affect the age distribution. We therefore consider the average age to be the best estimate for the deglaciation of Hans Island. In contrast, samples from Pim Island fall into two groups with mean ages more than five standard deviations apart. Four younger samples (Pim 1 in Table 1; three erratics and one bedrock) have a mean age of 10,400 $\pm$ 1,500 yr and a maximum age of 11,900 yr. Two other samples (Pim 2) have a mean age of 37,600 $\pm$ 5,200 yr. This latter age can best be explained by ^{36}Cl inherited from previous exposure to cosmic radiation. Both old samples are from the stoss (up-ice) sides of the summits of two roches moutonnées (protruding hillocks of bedrock), whereas the young bedrock sample is from the plucked lee side of another roche moutonnée. Briner and Swanson²⁴ have shown that erosion on the stoss side of glacially smoothed bedrock may be insufficient to remove previously accumulated cosmogenic nuclides, while erosion on the lee sides, subject to glacial plucking, is capable of removing inherited nuclides. We observe the same pattern for the bedrock samples from Pim Island. Hence, we consider the younger mean age (10,400 yr) to be our best estimate for the final deglaciation of the island.

Our ^{36}Cl ages demonstrate the coalescent Greenland and Ellesmere Island ice retreated from the Nares Strait about 10,000 yr ago. The ^{36}Cl ages are consistent with ^{14}C ages of 8,000 yr (8,800 cal. yr) for late-glacial deposits on the Greenland coast of the Nares Strait²⁵

and 9,000 yr (10,000 cal. yr) on the Ellesmere side¹⁰, and with the recent reconstruction of trunk ice retreat from Nares Strait based on ^{14}C -dated glaciomarine deposits²⁶. But qualitatively these two types of deglaciation records—one based on ^{14}C , the other on ^{36}Cl —are fundamentally different, and have different palaeoenvironmental implications. The ^{14}C -dated samples indicate when the coastal areas became deglaciated, but do not give information about how far into the strait the former ice extended. The ice could have filled the entire strait, or it could have stopped on the coasts leaving the open sea between Greenland and Ellesmere Island. In both cases, the coastal ^{14}C records would be similar. In contrast, the *in situ* ^{36}Cl data show unequivocally that the Nares Strait was filled with ice until $\sim$ 10,000 yr ago.

Maximum limiting age estimates for the beginning of the last glacial advance have been obtained from ^{14}C -dated, old marine shells redeposited in till and outwash on the east coast of Ellesmere Island. These ages range from $\sim$ 30,000 ^{14}C yr (ref. 16) ($\sim$ 36,000 cal. yr; ref. 22) on Cape Herschel, directly west of Pim Island, to 19,000–25,000 ^{14}C yr ($\sim$ 23,000–30,000 cal. yr) farther north and west²⁶. Taken together, the youngest of the old ^{14}C ages and our young ^{36}Cl ages constrain the age of the last deglaciation of the Nares Strait to have been between 23,000 and 10,000 cal. yr ago. During that period of full-glacial conditions, the connection between the Arctic and the Atlantic oceans via the Nares Strait was closed. This result demonstrates expansion of the Greenland ice sheet during the last glacial period, confirms the filling of the seaways in the high-latitude Arctic by ice during that time, and supports the model of the Innuitian ice sheet bridging the Laurentide and Greenland ice sheets. $\square$

Table 1 ^{36}Cl age determinations and chemical compositions

Sample	Material	³⁶ Cl/Cl (10 ⁻¹⁵)	Cl (p.p.m.)	B (p.p.m.)	Gd (p.p.m.)	U (p.p.m.)	Th (p.p.m.)	SiO ₂ (wt%)	TiO ₂ (wt%)	Al ₂ O ₃ (wt%)	Fe ₂ O ₃ (wt%)	MgO (wt%)	CaO (wt%)	MnO (wt%)	Na ₂ O (wt%)	K ₂ O (wt%)	P ₂ O ₅ (wt%)	³⁶ Cl age (kyr)		
																		e = 0	e = 5	
Delta. John Richardson Bay, 80.208° N, 71.020° W, 88 m.a.s.l., 8,050 ± 90 ¹⁴ C yr (ref. 23) = 9,100 cal. yr																				
EI92-20	EL_1.0	112 ± 11	274	25.5	2.5	0.6	1.4	4.96	0.03	1.20	0.27	1.26	50.6	0.01	0.01	0.87	0.07	8.6	8.6	
EI92-21	EL_1.0	120 ± 10	132	18.0	0.5	0.5	0.5	2.30	0.01	0.64	0.35	3.58	51.4	0.01	0.01	0.29	0.03	7.2	7.2	
EI92-22	EQ_1.0	58 ± 7	64	10.5	0.5	0.5	1.5	93.7	0.04	1.95	0.42	0.09	0.01	0.01	0.01	1.35	0.02	11.4	9.8	
EI92-23	EL_0.8	111 ± 13	137	20.5	1.0	0.9	5.2	35.4	0.16	4.58	0.79	2.40	29.8	0.01	0.01	3.47	0.10	9.0	9.0	
EI92-24	BL_	201 ± 25	106	26.5	1.0	0.7	1.7	9.94	0.08	2.44	0.90	1.60	47.3	0.01	0.01	0.82	0.07	9.8	9.9	
EI92-25	BL_	215 ± 21	95	17.0	1.5	0.6	1.0	10.6	0.04	1.48	0.57	1.40	48.7	0.01	0.01	0.72	0.05	10.3	10.4	
EI92-26	EQ_0.6	66 ± 9	29	3.5	0.5	0.5	1.7	97.3	0.06	0.53	0.07	0.06	0.01	0.01	0.01	0.32	0.02	12.0	9.6	
Average																		9.8 ± 1.7	9.2 ± 1.1	
Moraine. Hazen moraine, Hazen plateau, 81.867° N, 68.583° W, 510 m.a.s.l., 8,130 ± 200–6,995 ± 130 ¹⁴ C yr (refs 3, 21) = 9,200–7,800 cal. yr																				
EI93-46	ES_0.2	148 ± 16	41	(7)	(1)	1.1	5.3	74.4	0.28	5.25	1.67	1.28	8.09	0.03	0.66	1.13	0.10	8.0	7.8	
EI93-47	ES_0.5	181 ± 24	35	(7)	(1)	1.2	5.6	71.6	0.37	5.87	2.95	2.62	6.43	0.03	0.34	1.30	0.12	9.6	9.4	
EI93-48	ES_0.4	230 ± 42	37	(7)	(1)	1.2	5.6	62.1	0.36	6.83	2.61	2.22	11.8	0.04	0.84	1.59	0.11	8.8	8.8	
EI93-49	ES_0.3	184 ± 32	34	7.0	0.5	1.0	3.7	76.1	0.26	5.00	1.91	1.61	6.53	0.03	0.89	1.09	0.08	9.8	9.5	
EI93-30	ES_0.7	182 ± 14	44	(7)	(1)	3.0	10.0	64.7	0.52	7.55	2.83	3.10	8.68	0.04	1.30	1.63	0.14	8.5	8.3	
Average																		8.9 ± 0.8	8.8 ± 0.7	
Bedrock and erratics. Hans Island, 80.833° N, 66.583° W, ~150 m.a.s.l.																				
EI92-50	BLP	1015 ± 46	25	8	2	–	–	0.24	0.00	0.01	0.01	0.37	56.00	0.01	0.01	0.15	0.01	12.1	12.6	
EI92-61	EGP_0.8	76 ± 6	30	(15)	(5)	0.5	7.6	70.6	0.76	12.8	7.06	2.00	0.74	0.08	1.33	2.55	0.06	6.7	6.7	
EI92-52	EG_0.4	75 ± 7	141	85	4	2	12	74.2	0.21	13.4	1.25	30	0.70	0.03	3.91	4.63	0.07	9.8	9.1	
EI92-53	BLP	416 ± 39	44	(15)	(5)	0.5	0.5	0.36	0.00	0.06	0.19	0.80	56.20	0.01	0.01	0.04	0.01	8.5	8.7	
EI92-54	EG_1.0	77 ± 8	143	2	0.5	0.8	3.6	74.1	0.13	14.0	1.10	0.29	0.89	0.03	4.20	4.51	0.08	11.8	10.6	
EI92-55	EL_1.2	610 ± 60	29	(15)	(5)	0.5	0.5	0.27	0.00	0.23	0.06	0.97	55.80	0.01	0.01	0.10	0.02	8.6	8.9	
Average																		9.6 ± 2.1	9.4 ± 2.0	
Bedrock and erratics. Pim Island, 78.708° N, 74.250° W (within 1-km radius), elevations 320, 400, 410, 360, 320, 400 m.a.s.l.																				
EI96-71	EL_1.0	59.4 ± 4.5	577	13.5	2.0	0.7	0.5	4.11	0	0.62	1.63	19.7	28.90	0.08	0.02	0.37	0.01	8.5	7.9	
EI96-73	ED_0.4	138 ± 6	29	(15)	(3)	0.5	3.2	67.0	0.55	16.4	4.08	1.63	4.51	0.04	4.76	0.99	0.01	9.7	9.5	
EI96-74	EG_--	184 ± 7	24	(15)	(3)	0.5	3.2	70.3	0.35	16.0	2.83	0.62	3.65	0.03	4.83	1.31	0.01	11.3	11.1	
EI96-75	BGP	134 ± 7	68	20.5	2.5	0.8	3.3	75.6	0.08	13.7	0.94	0.03	0.71	0.01	3.53	5.07	0.09	11.9	11.6	
Average (Pim 1)																		10.4 ± 1.5	10.0 ± 1.7	
EI96-70	BGS	347 ± 15	74	14.5	3.5	0.9	11.0	73.8	0.15	14.2	1.19	0.15	1.31	0.01	3.60	4.97	0.07	33.9	32.5	
EI96-72	BGS	331 ± 11	101	12.0	3.5	1.9	19.0	74.4	0.18	13.9	1.76	0.26	1.26	0.01	3.57	4.45	0.06	41.2	37.4	
Average (Pim 2)																		37.6 ± 5.2	35.0 ± 3.5	

Nomenclature for samples analysed: first letter—Erratic, Bedrock; second letter—Limestone, Quartzite, Sandstone, Granite, Diorite, third letter—glacial Polish or Striae where present; numerical value for erratics—boulder height. For B and Gd, values in brackets indicate estimated concentrations used in the age calculations. Sample ages are calculated using erosion rates (*e*) of rock surfaces of 0 and 5 mm kyr^{−1}; for these erosion rates, two average surface ages are reported. Individual ages for *e* = 5 are younger than those for *e* = 0 for samples in which neutron activation of ^{35}Cl is an important mechanism of ^{36}Cl formation, and older for samples in which spallation of ^{39}K and ^{40}Ca dominates. Error of surface age is the larger of the internal (analytical uncertainty) and external (natural variability among samples) errors. Numbers in bold-face are our best estimates of surface ages; only these ages are discussed in text.

Methods

Sample preparation. Rock samples were collected from top horizontal surfaces of boulders and bedrock using hammer and chisel. The samples were cleaned of any organic material and carbonate crusts, crushed in a jaw crusher, and ground using a roller grinder. Size fraction 0.25–1.00 mm was leached in 3% nitric acid, rinsed in deionized water, and dried overnight at 100 °C. Silicates were dissolved in a hot mixture of hydrofluoric and nitric acids, carbonates were dissolved in cold diluted nitric acid, and Cl precipitated as AgCl. The AgCl was dissolved in concentrated NH₄OH. Sulphur was precipitated as BaSO₄ and separated from the liquid by centrifugation. The liquid was transferred and acidified using HNO₃ to re-precipitate AgCl. The purification step was repeated three times to minimize the amount of sulphur in the final AgCl. Chlorine-36 was determined on AgCl targets by accelerator mass spectrometry (AMS) at PRIME Laboratory, Purdue University. Aliquots of rocks were powdered and analysed for major elements by X-ray fluorescence spectrometry, for U and Th by neutron activation analysis, for B and Gd by neutron activation prompt gamma analysis (all at XRAL Laboratories, Don Mills, Ontario, Canada), and for Cl by an ion-specific electrode.

Age determination. Cosmogenic ³⁶Cl surface exposure ages were calculated using CHLOE software²⁷, with spallation production rates of 73.3 ± 4.9 atoms ³⁶Cl per g Ca yr⁻¹ and 154 ± 10 atoms ³⁶Cl per g K yr⁻¹ (ref. 20), and thermal neutron activation production rate calculated²⁸ from the fast neutron production rate of 586 ± 40 neutrons per g air yr⁻¹ (ref. 20). We used these production rates because: (1) they have been determined using a large number of samples of different ages and from 15 separate surfaces at eight locations (other production rates include fewer samples from fewer localities); (2) production rates from all three target elements (Cl, K and Ca) have been calculated simultaneously (other production rates have been determined for one or two elements); and (3) field and laboratory procedures used in the production-rate study and in this research were identical, which assures compatibility of all samples. Should the production rates change, the exposure ages can be recalculated using Table 1, which contains all the necessary data. The production rates used here are for sea level and high latitudes, and were scaled²⁹ to the locations of sample sites. It was not necessary to correct the production rates for temporal variability because at high latitudes the cosmic-ray intensity is constant. Likewise, the correction for seasonal snow cover is minimal in this relatively dry environment (precipitation rate, 50–100 mm yr⁻¹). All samples were collected above the Holocene marine limit (110–120 m), which means that they have been above sea level since deglaciation; therefore, no correction for water shielding was needed. Uncertainties of the ³⁶Cl ages (a combination of analytical errors and systematic errors associated with production-rate calculations) have been estimated to be <15% for studies in which multiple samples were analysed³⁰.

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The role of hydraulic fractures and intermediate-depth earthquakes in generating subduction-zone magmatism

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The presence of magmatism and intermediate-depth (70–300 km deep) seismicity at subduction zones is at first sight surprising. Magmatism is unexpected because the subduction of cool oceanic lithosphere makes these regions the coldest in the mantle. The current model for subduction-zone magmatism is that water released from the subducting slab enters the relatively warm mantle wedge, leading to a reduction in melting temperature and magmatism^{1–4}. But there is a problem with this scheme because it is thought that water cannot leave the slab by porous flow to enter the wedge. The occurrence of intermediate-depth earthquakes is surprising because of the inhibitory effect of the very high frictional stress on faults expected from the high pressure at these depths. One proposal put forward to explain intermediate-depth seismicity is that high pore-pressure might facilitate faulting by decreasing the friction^{5–7}. The hypothesis presented here is that non-percolating water provides the high pore-pressure, that the consequent faulting temporarily interconnects the water pores and, when a sufficient vertical height of water is interconnected, a hydrofracture is produced which transports the water out into the mantle wedge, thereby generating subduction-zone magmatism.

Hydrous minerals are formed in oceanic crust by hydrothermal circulation at mid-ocean ridges. This water is then released in subducting slabs by dehydration reactions due to increasing temperature and pressure encountered during subduction. A lateral transport mechanism has been proposed for moving water horizontally across the mantle away from the cold slab out to the hot mantle wedge. The water reacts with mantle peridotite forming

hydrous minerals. These are carried downward, parallel to the slab, as part of mantle wedge creep until they reach dehydration conditions. The released hydrous fluid phase moves vertically until it reaches mantle undersaturated in hydrated minerals, where it reacts forming hydrated minerals, repeating the cycle^{4,8}. The current explanation for most present-day subduction-zone magmatism is that this water, on reaching the hot mantle wedge, lowers the solidus, initiating melting.

There remains, however, a problem. How does water leave cold subducting oceanic crust to enter the mantle⁸? Equilibrium experiments undertaken to date suggest that the dihedral angle between water and mafic silicates at relatively low temperatures (<700 °C) and moderate pressures (<3 GPa) is greater than 60° (refs 9–11), and hence the fluid cannot form an interconnected network unless there is substantial porosity: for example, 3% at a dihedral angle of 70° (ref. 12). We add the caveat that our knowledge is incomplete because the permeability of the appropriate mineralogies has not yet been investigated at realistic conditions. Also, the influence of deformation is not known for certain, but given the rapid pace of textural equilibration it is probable that it is not important at geological strain rates. If the hydrous fluid and matrix are too cold to reach textural equilibrium, then the fluid is even less likely to percolate as it remains in the disconnected pockets in which it forms unless the matrix reaches very high porosity (3% porosity required if grains are approximately spherical, and 8% if the grains can be approximated by cubes)¹². So given our current knowledge, it is difficult to understand how a hydrous fluid could move by porous flow out from cold subducting slabs, into the mantle wedge, and (for example) initiate the lateral transport mechanism. Diffusion is much too slow a process to transfer water out into the mantle wedge⁸. In subducting slabs that are sufficiently hot to produce hydrous silicate melts, melt-borne water can reach the mantle wedge by porous flow, as textural equilibrium experiments show that melts interconnect through silicates¹³.

At depths of 70–300 km, the frictional stress across dry faults would be very high due to the high normal stress. Rocks would need to be unreasonably strong to generate sufficient shear stress to overcome such high frictional stresses to produce intermediate-depth earthquakes. The presence of non-percolating fluid in cold subducting slabs can facilitate generation of intermediate-depth earthquakes by increasing the pore pressure, reducing the effective normal stress, and hence also reducing frictional stress^{6,7}. The pore pressure following dehydration will approach lithostatic pressures. Higher pressures result if the dehydration rate is high and there is a net increase in volume^{14–16}. Ductile creep facilitated by dissolution-precipitation creep will bring the pore pressure towards lithostatic values, while slow conductive heating will further increase pore pressure¹⁷. Ductile creep will always be active and has been argued to be sufficiently rapid to raise pore pressures even in cool systems with finite permeability and negative volume change on dehydration¹⁸. Earthquakes could occur immediately on dehydration (dehydration embrittlement)^{19–21}, but this timing is not required. It is possible that the faulting occurs along old, well healed faults that were formed and hydrated before subduction^{7,20}.

Fracture under compression at low pressure has now been shown to result from the coalescence of dilating tensile microcracks²². The isolated pockets of water would nucleate microcracks, and the water would flow locally to hold open the microcracks and reduce the work required to dilate them under the higher confining pressure⁶. As the strain continues to increase, these microcracks would interact to give echelon crack arrays²³, and extend and coalesce (probably by propagating wing cracks²⁴, enhanced by water-mediated sub-critical crack growth). This would nucleate rupture, which would then propagate through the water-filled microcracks, leading to substantial slip, stress drop, and an earthquake. Thus, according to these theories of fracture and faulting, an earthquake would connect water-filled microcracks, and lead to increased porosity and permeability^{25,26}.

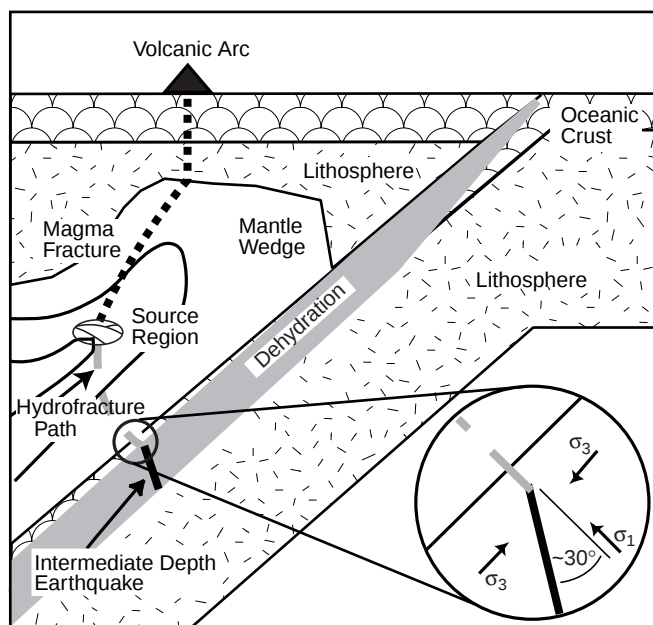


Figure 1 Diagram of proposed hypothesis. The grey region represents the location of dehydration processes. The curved dashed line represents the path of a hydrofracture, and the curved dotted line the path of a magma fracture. Here the hydrofracture has been shown reaching the hottest part of the mantle wedge where it generates rapid melting, leading to a fracture driven by magma. Hydrofractures could also stop in the cold regions of the wedge, initiating a lateral transport mechanism⁴. The direction of propagation of both fractures is controlled by the local stress field and is not vertical. This predicts that the ephemeral source region is horizontally farther away from the trench than is the volcanic front. The thin continuous lines represent hot isotherms in the mantle wedge. The inset is a magnified view of the oceanic crust, showing the possible relationship between the trace of the earthquake fault plane (thick black bar) and the hydrofracture direction. The direction of the greatest (σ_1) and least (σ_3) principal compressive stresses are assumed to be as shown, for the propagation directions presented.

When a large intermediate-depth earthquake occurs, it would allow a sufficient vertical height of water to interconnect on the fault plane, and initiate a hydrofracture at the upper end of the fault. There is extensive evidence for natural hydrofractures during devolatilization metamorphism²⁷. To generate a hydraulic fracture I assume in this model that sufficient fault permeability is developed to communicate the pressure and produce a stable hydrostatic gradient. It has been argued that faults can facilitate the movement of water in the crust via fault-valving²⁶, and also be high-permeability routes²⁸. Fluid-inclusion, oxygen-isotope and structural studies on Alpine eclogite facies rocks subducted to 40–70 km depth all support focused, pulsed fluid flow through fractures^{29–31}. In this hypothesis the free water is located as (1) isolated immobile pools at four-grain boundaries, (2) networks of water distributed along short, recently active fault planes (which could intersect forming arrays) or (3) be channelled into propagating hydrofractures following large intermediate-depth earthquakes.

In linear elastic fracture mechanics, a hydrofracture will propagate provided that the crack-tip critical stress intensity factor K is greater than the rock fracture toughness K_c (ref. 32). For a two-dimensional fluid-filled blade-like crack, $K = \Delta P h^{1/2}$, where the excess pressure $\Delta P = \Delta \rho g h$, h is the crack half height, $\Delta \rho$ is the density contrast, and g is the acceleration due to gravity. Experiments performed at room temperature and pressure have found that K_c is of the order of $1 \text{ MPa m}^{1/2}$ (ref. 32). Assuming the density contrast between the metamorphosed oceanic crust and the hydrous fluid to be $\Delta \rho = 2 \times 10^3 \text{ kg m}^{-3}$, we find $h > 15 \text{ m}$. Propagating cracks at initiation will have half-widths of $>0.02 \text{ mm}$; this calculation

uses width $w \approx 0.5(K_c/(\Delta\rho g M^3))^{1/3}$, where the elastic stiffness M is given by $\mu/(1-\nu)$, and μ is shear modulus and ν is Poisson's ratio³². High-pressure experiments indicate that K_c increases by at least a factor of 2 with pressure³³, while geological observations suggest that it could increase by as much as 100–1,000 (ref. 32). The critical height and width derived using values of K_c estimated from geological observations could therefore be much greater ($h > 300$ –1,500 m, $w > 10$ –200 mm), but the required vertical height of the interconnected water is less than the length of the rupture planes of large intermediate-depth earthquakes, which can be up to many tens of kilometres long.

Hydrofractures propagate perpendicular to the least compressive stress. Intermediate-depth earthquakes display a range of focal mechanisms, but many have their tension axis lying in the slab down-dip direction³⁴. For these earthquakes the least compressive stress will also be close to the slab down-dip direction. In such stress regimes the hydrofractures will propagate up and out, perpendicular to the slab surface (Fig. 1). Given simple corner flow for the mantle wedge, the stress regime and therefore the fracture propagation direction can be calculated; it has been shown to be in a direction up and out towards the hot part of the mantle wedge⁴ (Fig. 1).

The distance that a hydrofracture propagates will be related to its initial volume of water; this is because one of the factors limiting propagation will be loss of water from the fracture¹⁵, including loss by reaction with the wall rock. As different-magnitude earthquakes will interconnect fracture arrays containing different volumes of water, one can expect hydrofractures of various sizes that will propagate varying distances. The smallest might not leave the crust, while some might stop in the sediments, possibly leading to water saturated melting of the sediments. Melts of the sediments will react with the mantle wedge. Intermediate-sized hydraulic fractures will stop in the cold regions of the mantle wedge, reacting to form hydrated peridotite. In this case, the hydrous and sediment signature can reach the hot mantle wedge by the lateral transport mechanism described earlier⁴. If the rare, largest hydrofractures could propagate out to the hottest part of the mantle wedge, then they would rapidly form large volumes of melt. This can be expected to lead to the development of a fracture driven by magma, with the resulting rapid transport of the melt towards the near surface.

A detailed geochemical study of young lavas in the Marianas³⁵, including U-series disequilibria measurements which provide constraints on timescales of transport, makes a strong case for (1) slow transport of the sediment signature to the source region in a melt ($>350,000$ yr), (2) rapid transport of a hydrous fluid from the altered oceanic crust leading to melting ($<40,000$ yr), and (3) rapid movement of melt towards the surface from the top of the source region ($<8,000$ yr). The hypothesis I present here has the potential to explain the above observations, as it predicts a short timescale for movement by large hydrofractures and a long timescale for the transfer of slab geochemical signatures by the lateral transport mechanism.

My hypothesis is also relevant to one of the characteristic features of subduction-zone magmas: that they have a high ratio of large-ion lithophile elements to high-field-strength elements, thought to be caused by large-ion lithophile elements being preferentially carried by a hydrous fluid released from the subducting slab^{2,3,36}. If the water has time to interact with the mantle wedge then much of the large-ion lithophile element content would not be transported across the mantle wedge but would rather be advected away by the induced flow before they reached the source region³⁶. Hydrofractures could transport a significant amount of water very rapidly to the source region and circumvent this problem.

Magmatic output at the arc declines towards the back-arc from the peak at the volcanic front (a line defined by the stratovolcanoes nearest the trench). This decline could be understood if water for melting further from the volcanic front comes from the slab at

greater depths, where less water is released because most of it leaves earlier³⁷. This assumption would be consistent with my hypothesis as seismicity usually decays exponentially down to 300 km depth^{6,7}: the level of seismicity globally at 100 km depth is ~ 100 times greater than that at 300 km depth⁶.

Further tests for my hypothesis could include (1) rock mechanics tests to confirm how water helps initiate fractures at high confining pressure, (2) the prediction of major- and trace-element magma compositions and volumes, and (3) measuring the permeability of appropriate mineralogies to hydrous fluids at appropriate temperatures and pressures. Unfortunately, it would be impossible to detect directly a deep propagating hydrofracture, but it might be possible to detect associated double-couple earthquakes that are triggered by the passage of a hydrofracture³⁸. My hypothesis predicts concentrations of water in the oceanic crust which are too small to produce hydrofractures. This water content would lower seismic velocities, produce seismic anisotropy, and provide the potential to reflect high-frequency shear body-waves. In this context, I note that very high amplitude shear-wave reflections have been observed in studies in Japan, interpreted as being due to pools of fluid up to 0.5 km in radius (S. Sacks, personal communication). $\square$

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Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests

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Humans have altered global nitrogen cycling such that more atmospheric N₂ is being converted ('fixed') into biologically reactive forms by anthropogenic activities than by all natural processes combined¹. In particular, nitrogen oxides emitted during fuel combustion and ammonia volatilized as a result of intensive agriculture have increased atmospheric nitrogen inputs (mostly NO₃ and NH₄) to temperate forests in the Northern Hemisphere^{2–4}. Because tree growth in northern temperate

regions is typically nitrogen-limited⁵, increased nitrogen deposition could have the effect of attenuating rising atmospheric CO₂ by stimulating the accumulation of forest biomass. Forest inventories indicate that the carbon contents of northern forests have increased concurrently with nitrogen deposition since the 1950s^{6–8}. In addition, variations in atmospheric CO₂ indicate a globally significant carbon sink in northern mid-latitude forest regions^{9–12}. It is unclear, however, whether elevated nitrogen deposition or other factors are the primary cause of carbon sequestration in northern forests. Here we use evidence from ¹⁵N-tracer studies in nine forests to show that elevated nitrogen deposition is unlikely to be a major contributor to the putative CO₂ sink in forested northern temperate regions.

Although northern temperate forests might now function as significant CO₂ sinks, predicting their future role in the global carbon budget requires that causative factors for carbon sequestration be identified^{4,11–15}. Estimates of the effects of nitrogen deposition on forest carbon sequestration^{3,4,16,17} vary from 0.1 to 2.3 Pg carbon yr⁻¹. By comparison, the total terrestrial sink is currently 1.5–1.9 Pg yr⁻¹, with most CO₂ uptake occurring in northern mid-latitudes^{9,10}. If the higher estimates of the effects of nitrogen deposition on forest carbon uptake are accurate, then the terrestrial sink could persist well into the coming century as nitrogen deposition increases. Alternatively, if factors such as reforestation, changes in forest use and management, CO₂ fertilization, or nutrient transfers from soils to trees as a result of climate warming are mainly responsible for the terrestrial carbon sink, then rates of CO₂ uptake by temperate forests might follow different trajectories over the coming decades.

The role that nitrogen deposition plays in determining sink strengths of forests for CO₂ depends on where nitrogen inputs to forests ultimately reside^{12,18}. If the primary recipients are trees with woody tissues, high carbon-to-nitrogen (C:N) mass ratios (of between 200 and >500) and long turnover times, then the effects of nitrogen deposition on forest carbon uptake are relatively large. However, if nitrogen inputs are sequestered mainly in soils (C:N = 10–30) are exported as nitrate to drainage water or as nitrogen gases to the atmosphere, then the resultant carbon storage is minor.

Global analyses^{3,4} underscore the importance of determining the fates of nitrogen deposition in forests. The Townsend–Holland groups predicted global patterns of NO_y and NH_x deposition by combining estimates of NO_x and NH_x emissions with three-

Table 1 ¹⁵N-labelled nitrogen inputs to North American and European NITREX forests

Site	Location	Tree species	Ambient throughfall nitrogen (kg ha ⁻¹ yr ⁻¹)	¹⁵ N-forms added	¹⁵ N-labelled inputs* (kg ha ⁻¹ yr ⁻¹)
Bear Brooks (Maine, USA)	44° 52' N, 68° 06' W	Beech–maple–spruce (<i>Fagus grandifolia</i> , <i>Acer</i> sp., <i>Picea rubens</i>)	4	¹⁵ NO ₃ , plots ¹⁵ NH ₄ , watershed	32 and 60 on plots 29 on watershed
Harvard Forest (Massachusetts, USA)	42° 30' N, 72° 10' W	Oak (<i>Quercus velutina</i> , <i>Q. rubra</i>)	8	¹⁵ NH ₄ NO ₃ & NH ₄ ¹⁵ NO ₃	8 (ambient) 58 (fertilized)
Harvard Forest (Massachusetts, USA)	42° 30' N, 72° 10' E	Red pine (<i>Pinus resinosa</i>)	8	¹⁵ NH ₄ NO ₃ & NH ₄ ¹⁵ NO ₃	8 (ambient) 58 (fertilized)
Gårdsjön (Sweden)	58° 04' N, 12° 01' E	Norway spruce (<i>Picea abies</i>)	13	¹⁵ NH ₄ ¹⁵ NO ₃	49 (fertilized)
Aber (Wales, UK)	53° 12' N, 4° 00' W	Sitka spruce (<i>Picea sitchensis</i>)	15	¹⁵ NH ₄ ¹⁵ NO ₃ , also Na ¹⁵ NO ₃	35 (fertilized), 75 (fertilized, Na ¹⁵ NO ₃ only)
Alptal (Switzerland)	47° 03' N, 8° 24' E	Norway spruce (<i>Picea abies</i>)	17	¹⁵ NH ₄ ¹⁵ NO ₃	47 (fertilized)
Klosterhede (Denmark)	56° 29' N, 8° 24' E	Norway spruce (<i>Picea abies</i>)	20	¹⁵ NH ₄ ¹⁵ NO ₃	20 (ambient) 55 (fertilized)
Speuld (Netherlands)	52° 13' N, 5° 39' E	Douglas fir (<i>Pseudotsuga menziesii</i>)	50	¹⁵ NH ₄ (as (¹⁵ NH ₄) ₂ SO ₄)	50 (ambient) 4 (roof enclosures)
Ysselsteyn (Netherlands)	51° 30' N, 5° 55' E	Scots pine (<i>Pinus sylvestris</i>)	58	¹⁵ NH ₄ (as (¹⁵ NH ₄) ₂ SO ₄)	58 (ambient) 6 (roof enclosures)

* Labelled nitrogen inputs include throughfall nitrogen + fertilizer nitrogen, or, in the case of enclosures, filtered throughfall.

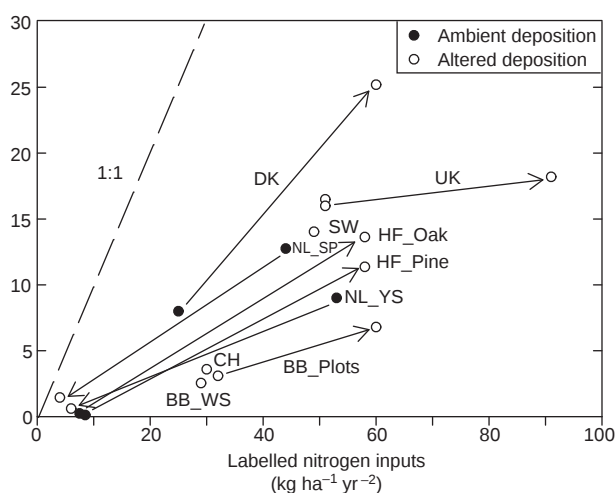


Figure 1 Uptake of ^{15}N -labelled throughfall by tree biomass after 1 to 3 years of tracer additions to non-fertilized (ambient deposition), fertilized and 'reverse fertilized' (throughfall nitrogen removed by means of roofs) plots in North America and Europe. Arrows show shifts in relationships at individual sites between ambient nitrogen deposition and either fertilized or reverse-fertilized conditions. Data for the Danish (DK), Welsh (UK), Dutch (NL_SP, Speuld; NL_YS, Ysselsteijn) sites are from ref. 19. Data from the Swiss (CH) and Swedish (SW) sites are from ref. 20 and O. J. Kjønaas (unpublished data), respectively. Data for Harvard Forest (HF_Pine, HF_Oak) and Bear Brooks (BB_WS, watershed study; BB_Plots, plot study) are from refs 21–23. Where $^{15}\text{NH}_4$ and $^{15}\text{NO}_3$ were applied separately, averages are shown. The masses of labelled nitrogen uptake by trees (y-axis) and labelled nitrogen inputs (x-axis) are linearly correlated. The least-squares regression is: $y = -0.87 + 0.25x$ ($r^2 = 0.68$, $P < 0.0001$).

dimensional atmospheric transport models ($\text{NO}_y = \text{HNO}_3 + \text{NO}_3^- + \text{HNO}_2 + \text{HO}_2\text{NO}_2 + \text{N}_2\text{O}_5 + \text{NO}_x + \text{PAN}$ (peroxyacetyl nitrate); $\text{NO}_x = \text{NO} + \text{NO}_2$; $\text{NH}_x = \text{NH}_4^+ + \text{NH}_3$). They then used spatially explicit estimates of nitrogen inputs and climate data as drivers for a process-based biogeochemical model (NDEP) to simulate ecosystem carbon dynamics globally at a $1^\circ \times 1^\circ$ scale. Their simulations predicted that CO_2 -carbon uptake due to NO_y deposition on the Earth's land surfaces currently ranges from 0.3 to 1.4 Pg yr^{-1} , and up to 2.0 Pg yr^{-1} with NH_x deposition included. Their simulations also indicate that most nitrogen deposition and carbon uptake resulting from this deposition in 1990 occurred between about 25°N and 55°N , mainly in temperate forests of eastern North America and Europe^{3,4}. The Townsend–Holland analyses allow for variations in the degree of ecosystem nitrogen retention (permitting system with high nitrogen deposition to retain smaller proportions of nitrogen inputs) and are highly sensitive to the proportion of nitrogen-induced plant growth that is allocated to woody tissues. Low estimates resulted when nitrogen

loss:input ratios increased with nitrogen deposition and when carbon allocation by plants to woody tissue was low (compared with foliage and roots). Their highest estimates resulted when trees took up 80% of nitrogen inputs and when proportional allocation of the resultant carbon fixation to woody tissues (compared with leaves and fine roots) was high.

We used results from ^{15}N -tracer studies conducted in six European and three North American forests (Table 1) to evaluate the potential of elevated nitrogen deposition to alter the sink strengths of temperate forests for atmospheric CO_2 . This set of experiments is unique in that tracers were applied to large plots (10^2 – 10^5 m^2) across one- to three-year intervals, either directly (without fertilizer, simulating ambient nitrogen inputs, and with chronic fertilizer applications), or in forest-canopy throughfall from which most NH_4^+ and NO_3^- ions were removed ('reverse-fertilization', using sub-canopy roofs and filtration). Tracing the movements of the ^{15}N additions through ecosystem components enabled us to estimate fluxes of N deposition (ambient, enhanced or diminished) into tree biomass and soil pools in a variety of forest types located along wide, natural and experimentally induced nitrogen-deposition gradients in the northern temperate region.

Our results indicate that trees are not the primary sinks for nitrogen deposition in these forests (Fig. 1). Across the entire nitrogen-deposition gradient, under both ambient and manipulated nitrogen inputs, the average tracer recovery was only 20% of one- to three-year ^{15}N additions. Within tree biomass, most tracers were recovered in leaves, fine roots, bark and twigs. The mean ^{15}N recovery in wood was 3% of inputs. The remaining fractions of applied tracers were recovered in litter, forest floor and mineral soil^{19–23}. Solution losses of labelled inputs in drainage were $<10\%$ of inputs at most sites. However, larger fractions of tracers were exported through drainage water at some European sites with high rates of nitrogen inputs^{19,24}. The two sites with the highest ambient rates of nitrogen deposition, Speuld and Ysselsteijn, lost 33% and 17% of ^{15}N tracers as dissolved inorganic nitrogen, respectively. Inorganic nitrogen losses at these sites were $\leq 10\%$ of inputs under reverse-fertilization. At Aber, in north Wales, 25–50% of applied tracers were lost as nitrate at the end of the labelling period, with the greatest losses occurring at inputs of $75 \text{ kg nitrogen ha}^{-1} \text{ yr}^{-1}$ (with NaNO_3 as the added nitrate form; $1 \text{ ha} = 10^4 \text{ m}^2$).

At the one- to three-year timescale of these tracer studies, most labelled nitrogen inputs to the forests entered the forest floor and soil pools, or, under high rates of nitrogen input, were exported as inorganic nitrogen. Thus, our results indicate that soil rather than tree biomass is the primary sink for NO_y and NH_x inputs to temperate forests. This is consistent with analyses of tree growth, which have not yet shown detectable increases in tree biomass accumulation with nitrogen deposition at our ^{15}N -labelled sites^{24–26}. If little accumulation of woody biomass results from chronic addition of atmospheric nitrogen to temperate forests, then elevated nitrogen deposition is not the primary contributor to the northern-

Table 2 Estimated influence of $\text{NO}_y + \text{NH}_x$ deposition on CO_2 -carbon uptake by forests

Forest ecosystem pool (1)	Nitrogen deposition allocated to pool (%) (2)	C:N (3)	Nitrogen deposition in pool (Tg) (= $5.1 \text{ Tg nitrogen} \times (2)$) (4)	CO_2 -carbon uptake (Pg) (= $(3) \times (4) \times 10^{-3}$) (5)
Non-woody biomass	15	25	0.77	0.019
Woody biomass	5	500	0.25	0.125
Soil (forest floor + mineral)	70	30	3.57	0.107
Leaching + gaseous losses	10	0	0.51	0.000
Totals	100		5.10	0.251

Percentages of nitrogen deposition allocated to ecosystem pools are estimated from US and NITREX ^{15}N -tracer studies as follows. Tracer-based estimates of nitrogen deposition taken up into tree biomass (leaves, fine roots, twigs, buds and wood + bark) averaged 20% across 9 sites and 18 treatments. The mean flux into woody biomass was 3% of labelled nitrogen inputs, but was conservatively estimated at 5%. C:N values were estimated as follows. Non-woody biomass (leaves, fine roots, twigs and buds) were assumed to contain 2% nitrogen and 48% carbon. Woody biomass was assumed to be 0.13% nitrogen (the mean concentration of bolewood from all 9 sites) and 48% carbon. Soil C:N was estimated to be 30, the high end of values reported for temperate forest floors for North America²⁷ and Europe²⁸. This is an upper limit as C:N ratios in forest floors subject to chronically high nitrogen deposition are as low as 20 and mineral soil values can be as low as 12. Losses of nitrogen (mostly as dissolved NO_3^- and possibly N_2O , NO and N_2 gases) can vary from <5 to 100% of inputs²⁹. We assumed relatively small losses. The 5.1-Tg estimate⁴ of nitrogen deposition on forests includes 3.1 Tg NO_y -nitrogen and 2.0 Tg NH_x -nitrogen deposition on forests, most of which currently falls on temperate forests in eastern North America, Europe and eastern Asia^{3,4}.

latitude CO₂ sink as suggested by modelling scenarios which assume that tree biomass accumulates 80% of atmospheric nitrogen inputs^{3,4}. To illustrate this point, we used our tracer results to construct a simple budget to estimate the effects of nitrogen deposition on carbon sequestration by temperate forests (Table 2). Our budget uses annual inputs of 5.1 Tg nitrogen (NO_y + NH_x) to the Earth's forests and the mean percentage assimilation of labelled nitrogen into non-woody biomass, wood and soils as measured in the 18 treatments at our 9 forest sites. We assumed that, on average, 10% of nitrogen inputs were exported as either dissolved NO₃⁻ or nitrogen gasses. Woody biomass constitutes the smallest nitrogen sink, but is the largest carbon sink owing to high C:N ratios. In contrast, although soil assimilates almost 15 times as much nitrogen deposition as wood, carbon sequestration in soil is slightly lower owing to low C:N ratios. Assuming that our budget is representative of patterns of nitrogen assimilation in temperate forests, we estimate that nitrogen deposition currently accounts for an uptake of 0.25 Pg carbon yr⁻¹ by forest. This value is between 6 and 8 times lower than the recent estimate⁴ of 1.5–2.0 Pg yr⁻¹ that is possibly attributable to nitrogen deposition on forests located predominantly in northern temperate regions.

Our budget (Table 2) is subject to some uncertainties. First, it applies results from temperate forest studies to forests in general. Patterns of assimilation of nitrogen inputs in tropical and boreal forests could differ from those in temperate forests. In particular, long-term fertilization studies in some boreal and sub-boreal forests suggest chronic, low-level nitrogen fertilization can lead to increased tree biomass²⁹. However, because temperate regions of eastern North America, Europe and eastern Asia currently receive nearly all of the 5.1 Tg yr⁻¹ of anthropogenic nitrogen inputs to the Earth's land surfaces, we feel that generalizing from the results of temperate forests is acceptable in the current climate. Too few tracer studies of nitrogen assimilation by boreal and tropical forests exist to determine how nitrogen deposition will be distributed between tree biomass and soils in these regions. Second, it is possible that the ¹⁵N-labelled inputs assimilated into tree biomass at our sites substituted to some extent for mineralized nitrogen that might otherwise have been taken up by trees. Thus, uptake of labelled nitrogen inputs does not necessarily lead to increased tree growth as assumed in our budget. Third, our budget assumes that tree and soil C:N ratios are fixed even though studies along nitrogen-deposition gradients indicate that tree tissue and upper soil horizon C:N ratios decrease under conditions of chronically elevated nitrogen deposition^{27,28}. If uptake of atmospherically deposited nitrogen substitutes for nitrogen mineralized in soil that would otherwise be taken up by trees, or if biomass or soil C:N ratios decrease with elevated nitrogen inputs, the effect of nitrogen deposition on carbon sequestration as estimated in Table 2 would be diminished. Therefore, our estimate of 0.25 Pg CO₂-carbon uptake yr⁻¹ resulting from nitrogen deposition is likely to be an upper limit.

In summary, despite some uncertainties, our results indicate that nitrogen deposition currently accounts for <20% of the annual 1.5–1.9 Pg CO₂-carbon uptake attributed to forest growth. Although recent budgetary and modelling analyses indicate that nitrogen deposition could account for an annual uptake of 0.1–2.3 Pg CO₂-carbon from the atmosphere^{1,3,4}, our analysis suggests that the effect of elevated nitrogen deposition on carbon uptake by forests is at the low end of this range of estimates. Other factors, such as long-term redistribution of nutrients from soils to trees, resulting from reforestation or climate warming, are more likely to be responsible for the putative carbon sink in northern mid-latitude forests. Finally, because rates of nitrogen deposition in boreal, subtropical and tropical regions are projected to increase in upcoming decades², it is important to determine whether the relationship we have observed between nitrogen deposition and carbon sequestration in temperate forests applies to forests in higher and low latitudes. □

Methods

Sites. The research was conducted in nine closed-canopy forests (dominant trees >35 years old at the start of treatments) in Europe and the northeastern United States (Table 1). The soils vary in texture, depth of forest floors and depth of solum, but all can be considered podzolic or gleyic. Ambient nitrogen deposition (throughfall ammonium plus nitrate) at the sites ranges from 4 to 58 kg ha⁻¹ yr⁻¹. Detailed descriptions are available elsewhere^{19–23}.

¹⁵N additions. In all cases, ¹⁵N tracers were added systematically to forest floors of large plots for at least an entire growing season. Two tracer experiments were conducted at Bear Brooks (Maine, USA). In the first, ¹⁵NO₃ was mixed with nitric acid applications (added at either 28 or 56 kg nitrogen ha⁻¹ yr⁻¹) to replicate 15 m × 15 m plots (*n* = 3) across three growing seasons (August 1988 through October 1991). In the second experiment, ¹⁵NH₄ was mixed with ammonium sulphate fertilizer additions (25 kg ha⁻¹ yr⁻¹) to a 10-ha watershed for two seasons (April 1991 to December 1992). Tracer experiments at the Harvard Forest (Massachusetts, USA) were conducted in a red pine forest and in an oak-dominated deciduous forest. In both forests, tracers were applied to single fertilized (50 kg nitrogen ha⁻¹ yr⁻¹ as NH₄NO₃ solutions) and non-fertilized plots (30 m × 30 m) in each forest type for two growing seasons (1991 and 1992). Each Harvard Forest plot was split with respect to ¹⁵N-ion additions, with one half of a lot (15 m × 30 m) receiving ¹⁵NH₄ and the other half receiving ¹⁵NO₃. The NITREX plot treatments included nitrogen fertilization (NH₄NO₃ at Aber, Alptal, Gårdsjön, Klosterhede and also NaNO₃ at two fertilization levels at Aber) and nitrogen removal from throughfall using sub-canopy roofs (Speuld, Ysselsteyn). ¹⁵N tracers were added to plots subject to ambient nitrogen deposition at Klosterhede, Speuld and Ysselsteyn. Tracers were added as ¹⁵NH₄ at Speuld and Ysselsteyn, both to ambient plots and beneath roofs. Both ¹⁵NH₄ and ¹⁵NO₃ were added together at the other NITREX plots, except for the NaNO₃-fertilized plots at Aber, which received only ¹⁵NO₃. NITREX plot sizes ranged from 100 to 1500 m² and tracers were added across a single calendar year (April or May 1992 to April or May 1993 at five sites; April 1995 to April 1966 at Alptal).

Tracer Recoveries. Analyses for ¹⁵N were conducted on samples collected before and at the end of tracer additions using isotope-ratio mass spectrometry³⁰. Although samples were collected shortly after the final tracer additions at most sites, samples collected 9 months after labelling were used for assessing tracer movements at three of the sites (Speuld, Ysselsteyn and Alptal). Recoveries of ¹⁵N tracers were estimated by mass balance³⁰.

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The *mahogany* protein is a receptor involved in suppression of obesity

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Genetic studies have shown that mutations within the *mahogany* locus¹ suppress the pleiotropic phenotypes, including obesity, of the *agouti-lethal-yellow* mutant^{2,3}. Here we identify the *mahogany* gene and its product; this study, to our knowledge, represents the first positional cloning of a suppressor gene in the mouse. Expression of the *mahogany* gene is broad; however, *in situ* hybridization analysis emphasizes the importance of its expression in the ventromedial hypothalamic nucleus, a region that is intimately involved in the regulation of body weight and feeding. We present new genetic studies that indicate that the *mahogany* locus does not suppress the obese phenotype of the melanocortin-4-receptor null allele⁴ or those of the monogenic obese models (*Lep^{db}*, *tub* and *Cpe^{fat}*). However, *mahogany* can suppress diet-induced obesity, the mechanism of which is likely to have implications for therapeutic intervention in common human obesity. The amino-acid sequence of the *mahogany* protein suggests that it is a large, single-transmembrane-domain receptor-like molecule, with a short cytoplasmic tail containing a site that is conserved between *Caenorhabditis elegans* and mammals. We

propose two potential, alternative modes of action for *mahogany*: one draws parallels with the mechanism of action of low-affinity proteoglycan receptors such as fibroblast growth factor and transforming growth factor- β , and the other suggests that *mahogany* itself is a signalling receptor.

Signalling from α -melanocyte-stimulating factor (α -MSH) through the melanocortin receptors *Mclr* and *Mc4r* regulates pigmentation and body weight, respectively. Overexpression of agouti, an antagonist of *Mclr* and *Mc4r* (refs 5, 6), results in yellow, obese mice. The murine *mahogany* gene (*mg*) acts in a dosage-dependent manner within the agouti pathway to compensate for agouti overexpression and for lack of signalling from an *Mclr* null allele^{2,3,7}. Mice homozygous for both *mg* and a null allele of *Mclr* (recessive yellow, *Mclr^e*) are yellow, as are *Mclr^e/Mclr^e* mice, indicating that *mg* does not act downstream of *Mclr* (ref. 2). We performed a similar experiment with obese *Mc4r* (ref. 4) knockout mice (Fig. 1). For both sexes, all the animals homozygous for the *Mc4r* null allele were roughly equally obese and were heavier than wild-type mice, independently of the *mg* genotype. These data strengthen and confirm the data obtained with *Mclr* knockouts, strongly indicating that *mg* acts at or upstream of both melanocortin receptors.

It has been assumed that *mg* acts specifically within the agouti pathway. We tested this by asking whether *mg* can suppress the obesity of other monogenic obese mouse mutants and whether it can suppress diet-induced obesity. We set up appropriate genetic crosses to produce mice that segregated *mg* and one of the mouse obesity mutations, *Cpe^{fat}*, *tub*, or *Lep^{db}*. No suppression of obesity by *mg* was seen for any of the monogenic obese mutants (Fig. 2a–c), lending credence to the assumed specificity of action of *mg* within the agouti pathway. To determine whether *mg* can suppress diet-induced obesity, we weaned C3HeB/FeJ-*mg^{3J}* and C3H/HeJ mice onto either normal chow (physiological fuel value (PFV) 3.63 kcal g⁻¹ with 9% fat) or a high-fat diet (PFV 4.53 kcal g⁻¹ with 42.2% fat). Converting the grams of food consumed to calories indicated that male C3H/HeJ mice fed on normal chow or the high-fat diet consumed ~97 kcal per week and 96 kcal per week, respectively; male C3HeB/FeJ-*mg^{3J}* mice fed on normal chow or the high-fat diet consumed ~83 kcal per week and ~81 kcal per week, respectively. Despite the equal calorie intake, the C3H/HeJ mice on the high-fat diet gained more weight than the C3H/HeJ mice fed on normal chow ($P=0.0004$). In contrast, the C3HeB/FeJ-*mg^{3J}* mice on either diet showed no statistically significant difference in weight (Fig. 2d). Female data (not shown) showed the same trends, although there

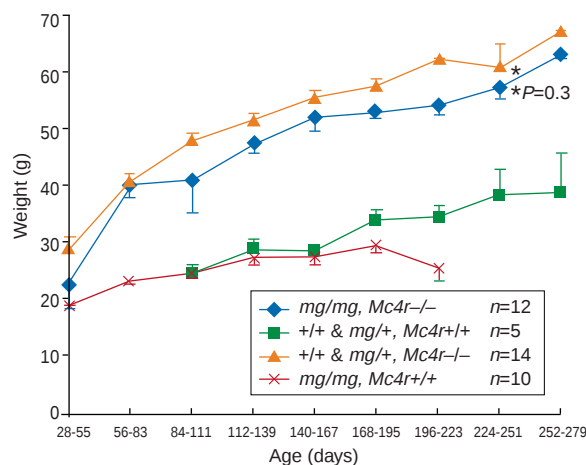


Figure 1 Effect of *mg* on *Mc4r^{-/-}*-induced weight gain in females. Values shown are the means $\pm$ s.d. within a designated time interval. We performed a *t*-test on the weights of *mg/mg*, *Mc4r^{-/-}* animals and *+/+*, *Mc4r^{-/-}* or *mg/+*, *Mc4r^{-/-}* animals at days 224–251 and found no statistically significant differences in either the female or the male (data not shown) data.

was no statistical significance between any of the mice on either diet.

To identify the *mg* gene we undertook a positional cloning strategy. Multiple genetic crosses produced second-generation mice ($n=2437$) segregating *mg* that were used to localize the *mg* locus genetically to ~ 0.6 centimorgans (cM) (Fig. 3b). We created a 1 megabase contig (Fig. 3c) around the *mg* locus containing 30 bacterial artificial chromosomes (BACs), most of which were made into random sheared libraries for shotgun sequencing. At the end of this project we estimated 85% sequence coverage across this ~ 0.6 -cM interval and believe that we have found all genes within the region. We identified 29 genes, 15 of which are novel (Fig. 3d). Within the final minimal interval for *mg*, indicated by a purple line in Fig. 3, there were 11 genes, 9 of which were unknown. We tested all 11 as candidates for *mg* by examining the three mutant alleles of the *mahogany* locus, the original allele, *mg*, that arose in a stock of Swiss $\times$ C3H mice, and two alleles that arose independently on the C3H background (C3HeB/FeJ-*mg*^{3J}/*mg*^{3J} and C3H/He-*mg*^L/*mg*^L) by northern blot analysis, Southern blot analysis, reverse transcription polymerase chain reaction (RT-PCR) and single-strand conformation polymorphism (SSCP) analysis of genomic PCR products designed to cover the intron-exon boundaries of most of each gene. In all, we analysed 20 genes in this way, one of which showed differences between the wild-type and mutant alleles on a northern blot (Fig. 4a).

The wild-type expression pattern of this gene gives three bands of size ~ 9 kilobases (kb), 4.5 kb and 3.8 kb, of which the largest is the most prominent (Fig. 4a). The smaller two can be seen in all tissues, but, depending on the tissue, may require extended exposure. Each of the different *mg* alleles gave a different expression pattern. For further clarification of genetic marker order we mapped many of the

STSs and ESTs to the Millennium BSB ((C57BL/6J $\times$ SPRET/Eij) $\times$ C57BL/6J) mapping panel²⁶ (Fig. 3a). C3HeB/FeJ-*mg*^{3J}/*mg*^{3J} has extremely low expression; the 9-kb message only appeared, very faintly, in brain, hypothalamus and fat on northern blots. C3H/He-*mg*^L/*mg*^L expresses a single aberrant band of ~ 9.5 –10 kb in kidney, heart, muscle, fat and, most prominently, in the brain and hypothalamus. LDJ/Le-*mg*/*mg* shows an altered ratio of the three wild-type messages: the 9-kb message is reduced whereas the two smaller messages are more highly expressed, being very abundant in fat and hypothalamus in particular. *In situ* analysis of wild-type brain shows that *mg* is expressed throughout, notably in the hippocampus. Low-power (magnification $\times 200$) examination of *mg* hybridization to wild-type (Fig. 4b, top panel) and LDJ/Le-*mg*/*mg* (data not shown) brains appears equivalent, whereas hybridization to C3HeB/FeJ-*mg*^{3J}/*mg*^{3J} brain shows an overall reduction of expression in all regions (data not shown), consistent with the northern blot data. High-power ($\times 400$) evaluation of the hypothalamic region revealed an allele-specific reduction of expression in the ventromedial hypothalamic nucleus (VMH) of LDJ/Le-*mg*/*mg* brains (Fig. 4b, LDJ/Le-*mg* VMH image) compared with wild-type brains (Fig. 4b, C3H/HeJ VMH image). Although the VMH is not the only brain region that shows *mahogany* expression, it is the only place in which differential expression in mutant alleles was observed. The altered pattern of expression seen in the VMH of LDJ/Le-*mg*/*mg* mice is particularly interesting, as many neuropeptides and receptors known to be involved in body-weight regulation are expressed there, including *Mc4r* (ref. 8).

We originally identified two overlapping mouse complementary DNAs of 1,051 base pairs (bp) and 2,419 bp. We used these cDNAs as a starting point and, by using both the public expressed sequence

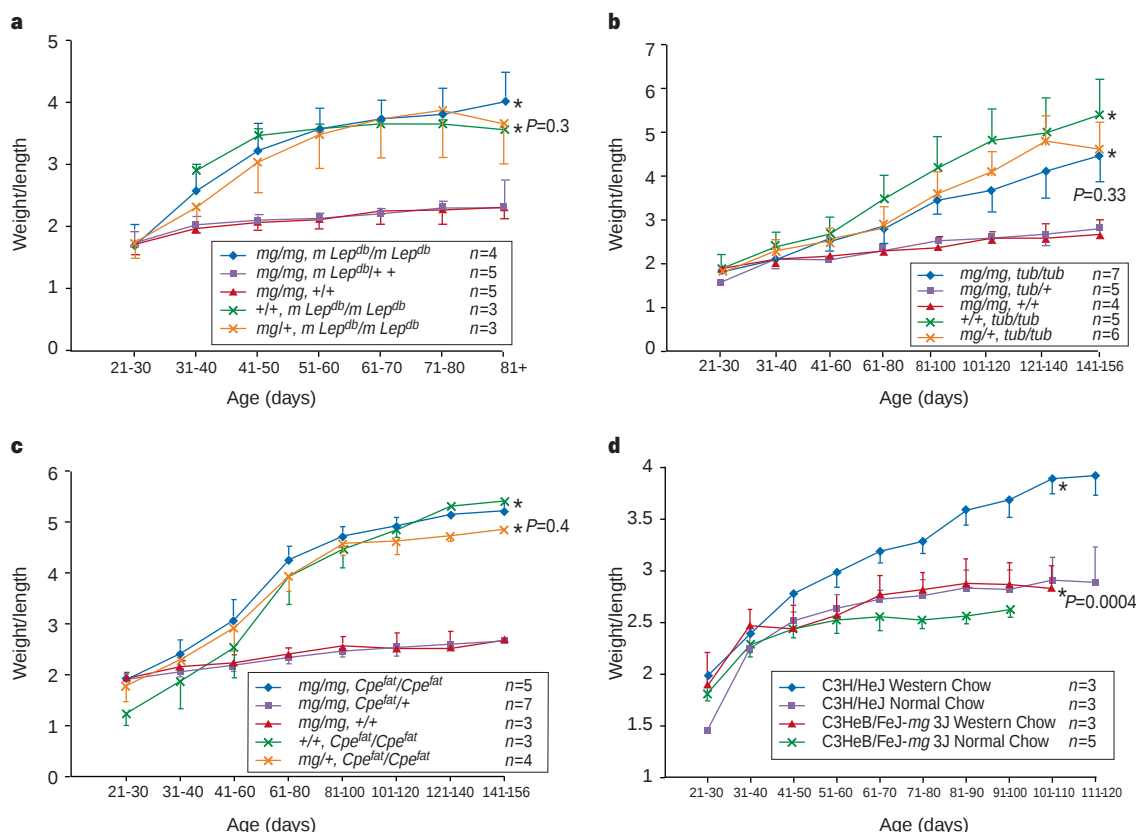


Figure 2 Effect of *mg* on the monogenic obese mutants *tub*, *Cpe*^{fat} and *Lep*^{db} and on obesity induced by a high-fat diet. The values depicted are the means $\pm$ s.d. of weight:length ratios. **a–c**, The affect of *mg* on **a**, *tub*, **b**, *Cpe*^{fat} and **c**, *Lep*^{db} mutations in females. We performed *t*-tests on the weight:length ratios of the mice that were genotypically *mg*/*mg*, mutant/mutant versus *+/+*, mutant/mutant (indicated by asterisks). No statistically significant differences were found in

either the female or the male (data not shown) data. **d**, Effect of *mg* on diet-induced obesity. Male data are shown. A *t*-test was performed on the weight:length ratios of animals in the C3H/HeJ and C3HeB/FeJ-*mg*^{3J} classes (indicated by asterisks) fed a western diet at days 101–110 and a statistically significant difference was found, $P=0.0004$.

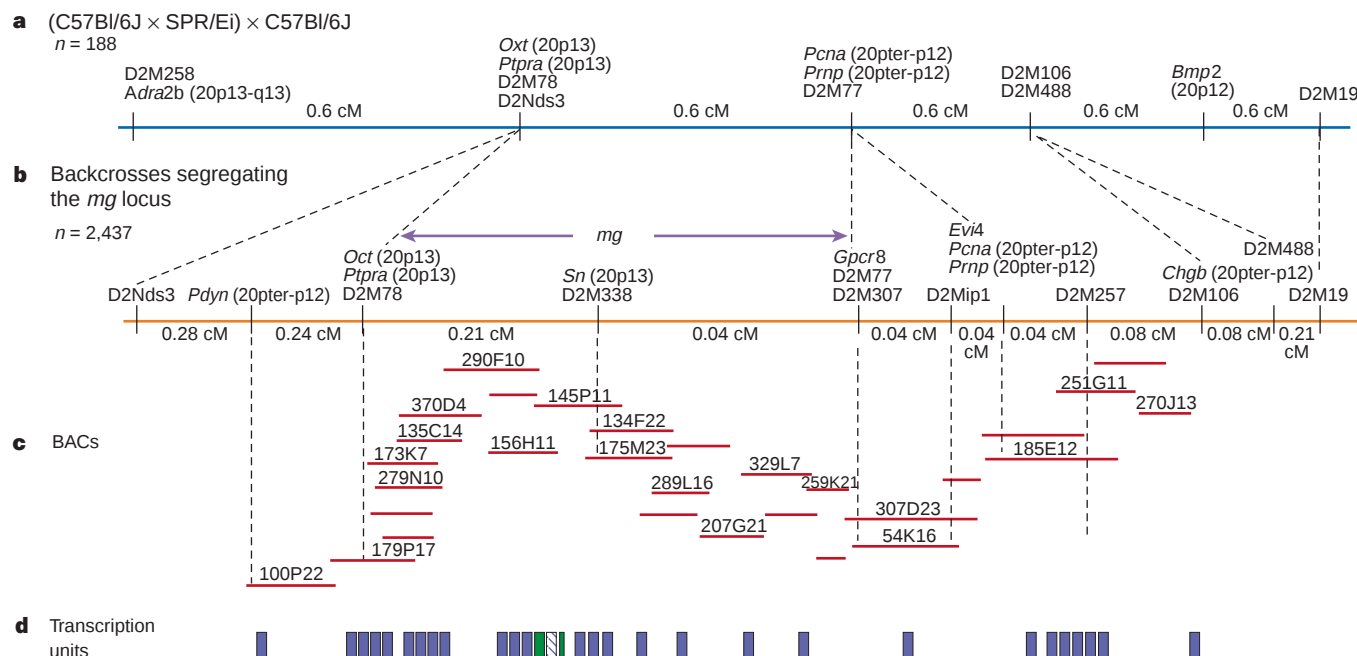


Figure 3 Genetic and physical map of the region surrounding the *mg* locus. All Mit markers are presented with shortened names; for example, *D2Mit77* is shown as *D2M77*. For loci also mapped on the human cytogenetic map, the location is indicated in parentheses after the gene symbol. Genetic distances are given in cM. **a**, The genetic map of the *mg* gene region on the Millennium BSB mapping panel²⁶. **b**, The genetic map obtained from crosses segregating *mg* mutant alleles. Relative positions of loci mapped in both the BSB panel and the *mg* segregating crosses are indicated by dashed lines. **c**, The ~1-megabase BAC contig across the *mg* gene region of chromosome 2. BACs used to construct random-sheared libraries for sequencing are labelled with their full name, and are

represented by lines that are roughly proportional to their physical size and positioned relative to their physical overlaps. **d**, Transcription units identified here. Each box represents an individual transcription unit. The filled green box identifies the *mg* gene. The hatched box represents a member of the high-mobility-group gene family and sits between coding exons 21 and 22 of the *mg* gene. The map location of the human orthologue of *mg* was determined using both the Genbridge²⁷ and the G3 (ref. 28) radiation hybrid panels. *mg* maps to human chromosome region 20p13, as predicted, and does not segregate with D20S752.

tag (EST) database and our own, as well as by identifying two cDNA clones from a human liver library, we have been able to build over 7,990 bp of human sequence. The derived human sequence allowed us to estimate the intron–exon boundaries within the mouse genomic sequence; these boundaries were verified by PCR amplification and sequencing. In total we have 6,487 bp of mouse sequence. The mouse genomic locus spans over 160 kb and has 30 identified exons, at least one of which is differentially spliced.

We sequenced the mutant alleles, checking all intron–exon boundaries. A 5-bp deletion at nucleotide 2,809 was found in the coding sequence of the *mg* gene from C3HeB/FeJ-*mg*^{3j}/*mg*^{3j}; this deletion introduces a stop codon at codon 937 of the sequence, two codons 3' of the deletion, resulting in a severely truncated protein lacking many interesting domains (see below). We have not yet identified primary DNA mutations for the other alleles. The combined northern blot analysis, *in situ* hybridization analysis and sequence analysis of the mutant *mg*^{3j} allele led us to believe that we have identified the mouse *mahogany* gene.

The 4,011-bp open reading frame (ORF) of mouse *mg* predicts a 1,336-amino-acid polypeptide with a relative molecular mass of 148,706. BLAST searches of the NCBI and SwissProt protein databases identified two human paralogues with a similar modular architecture (KIAA0534, GenBank accession number 3043592, and MEGF8, GenBank accession number AB011541), as well as a *C. elegans* homologue (YC81_CAEEL, GenBank accession number Q19981) (Fig. 5a).

The SMART⁹ domain tool revealed sequence motifs in the mahogany polypeptide that provide further clues to its biological function (Fig. 5b). The single-transmembrane-domain mahogany protein has a large extracellular sequence of 1,289 amino acids, which contains three epidermal growth factor (EGF) domains¹⁰, two laminin-like EGF repeats, a CUB domain¹¹, two plexin-like

repeats¹², a C-type lectin^{13,14} and seven consecutive Kelch repeats¹⁵ (Fig. 5b). Multiple EGF domains are commonly found in type I membrane proteins (membrane proteins with their amino terminus in the cytosol) that are involved in cell adhesion and receptor–ligand interactions⁹. Laminin-like EGF modules are found in several proteoglycans such as perlecan and heparin sulphate proteoglycan. As CUB domains also occur frequently in glycosylated proteins and as C-type lectins bind carbohydrate, we predict that mahogany is heavily glycosylated and that carbohydrate interactions are essential for the function of mahogany.

Many Kelch-motif-containing proteins have been found that, like mahogany, have multiple consecutive domains. Such consecutive four-stranded β -sheets Kelch motifs form a bladed β -‘propeller fold’ that is common in many sialidases and other enzymes¹⁴. Unlike other well-recognized domains, the ‘plexin’ repeat is less well defined. It was first recognized as a triple repeat in the *Xenopus* protein plexin, which shows similarity to the proto-oncoprotein MET¹². Since then, this cysteine-rich repeat has been found in six MET gene family members¹¹, three of which signal through tyrosine kinase and three of which are proposed to have signalling function through a new conserved cytoplasmic domain. The cytoplasmic tail of mahogany is short (126 amino acids) and has no previously defined signalling domain. However, an 8-amino-acid stretch is conserved in proteins (Fig. 5a) from human, mouse and *C. elegans*. The conservation of sequence across such widely evolutionary divergent species strongly indicates that it is a functional domain, possibly a signalling motif.

The multidomain structure of mahogany is complex but shows many similarities to receptor and receptor-like proteins. We predict that mahogany is a large, membrane-spanning protein, with multiple extracellular domains that may have a binding or gathering function as well as a highly conserved putative signalling motif in

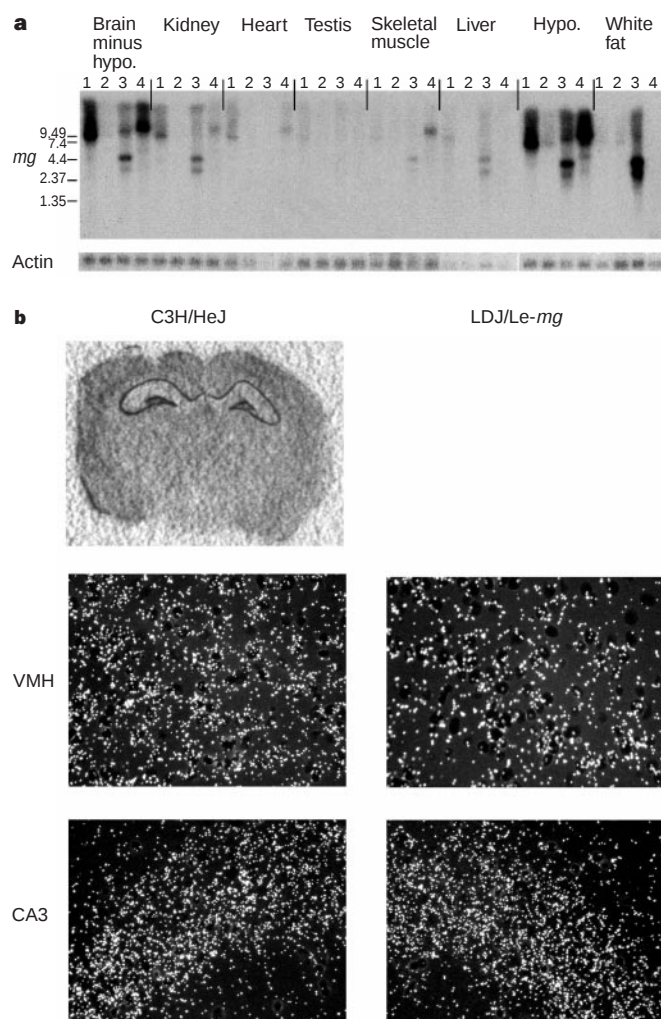


Figure 4 Expression patterns of *mg*. **a**, Northern blot analysis of *mg*, showing expression of C3H/HeJ (lane 1) and the three mutant alleles of *mg*: C3HeB/FeJ-*mg*^{3J} (lane 2), LDJ/Le-*mg*^L (lane 3) and C3H/HeJ-*mg*^L (lane 4). Size markers are on the left. Hybridization with actin is shown below as a loading comparison. **b**, Altered expression of *mg* in the neurons of the VMH. Top panel, an antisense autoradiographic image of a C3H/HeJ brain at the level of the third ventricle, showing that *mg* is expressed widely throughout the mouse brain (original magnification $\times 200$). Bottom panels, further evaluation of selected antisense dark-field images show decreased *mg* expression in LDJ/Le-*mg* mutants compared with in C3H/HeJ brain in the region of the VMH but not in the CA3 region of the brain (original magnification $\times 400$).

the cytoplasmic tail. It is possible that mahogany acts as an accessory receptor, gathering extracellular molecules that are presented to high-affinity receptors, which subsequently signal. Examples of such accessory receptors include the transforming growth factor- β betaglycan receptor, the insulin growth factor-2 receptor and the proteoglycan fibroblast growth factor receptor¹⁶. It is unclear whether the similarity of mahogany to proteoglycans is meaningful in this regard. Genetic and biological evidence indicates that mahogany probably cannot act to present ligand to the melanocortin receptors, as this would be dichotomous with the antagonism of these receptors by agouti and agouti-related protein^{17,18}. Instead, mahogany could present the antagonists to the receptors, thereby reducing signalling. Reduced expression of mahogany would result in increased signalling. An alternative model could simply be that mahogany sequesters away the ligand, effectively increasing the concentration of antagonists in the environment. A similar mechanism of sequestering leptin away from the leptin receptor, thereby reducing signalling and downstream production of proopio-

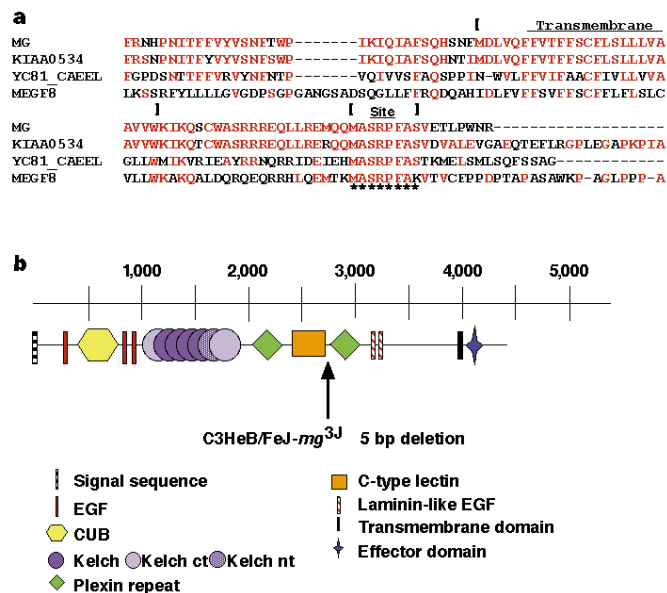


Figure 5 Domains of the mahogany protein. **a**, Alignment of the mahogany protein sequence with its family members (see text), showing the transmembrane domain and cytoplasmic tail. The alignment was performed using CLUSTAL W version 1.5 (ref. 29). Residues highlighted in red indicate conservation between at least two of the four proteins shown. The transmembrane region is indicated within the brackets. The site conserved among all four proteins is indicated by asterisks. KIAA0534 and MEGF8, two human paralogues; YC81-CAEEL, a *C. elegans* homologue. **b**, The molecular modular architecture of the mahogany protein. Upon folding of the protein the two Kelch units annotated as ct form the C-terminal (ct) Kelch domain. The Kelch domain annotated as nt is the N-terminal Kelch domain in the folded protein.

melanocortin products, including α -MSH, is also feasible, yet less likely. The identification of the *mahogany* protein is a major first step towards a better understanding of its role in the agouti pathway, in the reported hyperphagia and hypermetabolic behaviour of *mg* mutants³ and in suppressing diet-induced obesity, and may open the way to new therapeutic intervention in common human obesity. **Note added in proof:** Since the submission of this manuscript the sequence of the *mg* gene has been published by another group under the name attractin. Attractin is described as a haematopoietic-specific, secreted T-cell activated protein³⁰. We have also, since submission of this manuscript, identified alternative splice forms of the *mg* gene, two of which constitute secreted forms. □

Methods

Weight analysis. All animals were multiply housed, but segregated by sex and genotype, and were fed a 9% fat chow diet *ad libitum*, except as noted. Animals were weighed and their lengths were measured every ten days, unless otherwise stated. A weight:length ratio for each animal was generated at each time point.

Mc4r^{-/-} (ref. 7) males and LDJ/Le-*mg*/*mg* females were mated. Resulting F₁ mice were intercrossed, and the F₂ mice were genotyped for the *Mc4r* knockout locus and assayed for coat colour. *mg* heterozygotes could not be distinguished from wild-type mice and were combined into a single group for these analyses. Animals were weighed every 20 days. A Student's *t*-test was performed on the weights of animals that were genotypically *mg*/*mg*, *Mc4r*^{-/-} and on *+/+*, *Mc4r*^{-/-} or *mg*/*+*, *Mc4r*^{-/-} animals at days 224–251. To measure the effect of *mg* on monogenic obese mutants, genetic crosses between *tub*, *Cpe*^{fat}, and *Lepr*^{db} animals were performed to produce mice carrying 0, 1 or 2 copies of *mg* with 0, 1 or 2 copies of each of the mutant alleles. The parental strains for these crosses were C57BL/6J-*tub*/*tub* and LDJ/Le-*mg*/*mg*, C57BL/KsJ-*fat*/*+* and LDJ/Le-*mg*/*mg*, and C57BL/KsJ-*Lepr*^{db}/*+* and LDJ/Le-*mg*/*mg*. We performed *t*-tests on the weight:length ratios of the mice that were genotypically *mg*/*mg*, *mutant*/*mutant* versus *+/+*, *mutant*/*mutant*.

To assess the affect of *mg* on obesity induced by a high-fat diet, we fed C3H/

HeJ and C3HeB/FeJ-*mg*^{3J} siblings *ad libitum* on either a 9% fat chow diet or a high-fat diet (42.2% fat), formula TD88137 (Harlan Teklad, Madison, Wisconsin). We performed a *t*-test on the weight:length ratios of animals in the C3H/HeJ and C3HeB/FeJ-*mg*^{3J} classes fed a western diet at days 101–110 and found a statistically significant difference, *P*=0.0004.

Genetic mapping. The crosses and the number of animals for each (*n*) were (LDJ/Le-*mg*^{3J} × CAST/Ei) × LDJ/Le-*mg*^{3J} (*n*=1,588), (C3HeB/FeJ-*mg*^{3J}/*mg*^{3J} × CAST/Ei) × C3HeB/FeJ-*mg*^{3J}/*mg*^{3J} (*n*=324), (C3HeB/FeJ-*mg*^{3J}/*mg*^{3J} × MOLF/Ei) × C3HeB/FeJ-*mg*^{3J}/*mg*^{3J} (*n*=216) and (C3HeB/FeJ-*mg*^{3J}/*mg*^{3J} × C57BL/6J) × C3HeB/FeJ-*mg*^{3J}/*mg*^{3J} (*n*=309). The 2,437 F₂ mice were analysed by coat colour to determine their genotype at the *mg* locus. As mice change colour slightly at each hair moult and because the phenotype differences between *mg*/*mg* versus *mg*/+ mice can be subtle, all mice were phenotyped at the same age by a single person. Genomic DNA was analysed for multiple simple sequence length repeat polymorphism (SSLP) markers¹⁹. With the first ~100 mice we determined that *mg* mapped roughly 15 cM proximal to *agouti* between markers D2Mit19 and D2Nds3 (Fig. 3). All remaining animals were genotyped for these markers. Animals recombinant in that interval were typed with all available Mit markers between and for the ever-growing number of markers developed during the project, totalling 265 markers. They are available as a list of PCR primers from the authors.

Sequence analysis. More than 36,000 individual sequences from the region were compared by BLAST²⁰ with sequences from publicly available databases and were analysed using GRAIL²¹ to identify potential coding sequences. In addition, sequences from overlapping BACs were assembled using PHRAP^{22–24} and the resulting contigs were also analysed using BLAST and GRAIL to aid in gene prediction. These data were displayed in ACEDb (data available from ncbi.nlm.nih.gov) to visualize predicted exons and their relationships to each other.

Northern blot analysis. Poly(A)⁺ RNA was extracted from the tissues indicated from wild-type, C3H/HeJ, C3HeB/FeJ-*mg*^{3J}, LDJ/Le-*mg* and C3H/HeJ-*mg*^L mice, according to the manufacturer's instructions. RNA STAT-60 (Tel-Test Inc., Friendswood, Texas) was used to isolate total RNA. Poly(A)⁺ RNA was isolated using the Poly(A)PureTM messenger RNA purification kit (Ambion Inc., Austin, Texas). 2 µg of each mRNA was separated on a 1% agarose-formaldehyde gel, transferred to nylon and hybridized with a probe for *mg* corresponding to nucleotides 990–1,406 of the murine cDNA sequence, using Rapid-hyb Buffer (Amersham). Filters were washed with 0.1 × SSC (1.5 mM trisodium citrate, 15 mM sodium chloride, pH 7.0), 0.1% SDS and exposed to Kodak X-omat film overnight.

In situ hybridization analysis. Complementary RNA sense and antisense ³⁵S-radiolabelled (10⁷ c.p.m.) probes corresponding to nucleotides 677–4,079 of the murine *mg* cDNA sequence were hybridized to 10-µm brain whole-mount coronal sections of C3H/HeJ, LDJ/Le-*mg* and C3HeB/FeJ-*mg*^{3J} animals²⁵. Localization of mRNA transcripts was detected by dipping slides in Kodak NBT-2 photoemulsion and exposing for 16 days at 4 °C, followed by development with Kodak Dektol developer. Slides were counterstained with haematoxylin and eosin and photographed. Controls for the *in situ* hybridization experiments included the use of a sense probe, which showed no signal above background levels.

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The mouse *mahogany* locus encodes a transmembrane form of human attractin

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Agouti protein and agouti-related protein are homologous paracrine signalling molecules that normally regulate hair colour and body weight, respectively, by antagonizing signalling through melanocortin receptors^{1–7}. Expression of *Agouti* is normally limited to the skin, but rare alleles from which *Agouti* is expressed ubiquitously, such as *lethal yellow*, have pleiotropic effects that include a yellow coat, obesity, increased linear growth, and immune defects^{8–11}. The *mahogany* (*mg*) mutation suppresses the effects of *lethal yellow* on pigmentation and body weight, and results of our previous genetic studies place *mg* downstream of transcription of *Agouti* but upstream of melanocortin receptors¹². Here we use positional cloning to identify a candidate gene for *mahogany*, *Mgca*. The predicted protein encoded by *Mgca* is a 1,428-amino-acid, single-transmembrane-domain protein that is expressed in many tissues, including pigment cells and the hypothalamus. The extracellular domain of the *Mgca* protein is the orthologue of human attractin, a circulating molecule produced by activated T cells that has been implicated in

immune-cell interactions^{13,14}. These observations provide new insight into the regulation of energy metabolism and indicate a molecular basis for crosstalk between melanocortin-receptor signalling and immune function.

Agouti protein signals hair-follicle melanocytes to synthesize yellow pigment, pheomelanin, instead of eumelanin, a black or brown pigment. Animals that carry the most common *Agouti* allele, *A*, exhibit individual hairs that are mainly black or brown, but contain a subapical band of yellow pigment caused by transient expression of *Agouti* during hair growth¹⁵. Homozygosity for *mg* greatly reduces the width of the yellow band (Fig. 1c); heterozygosity has a subtle effect that is most easily detectable on a *lethal-yellow* (*A^y*) background^{12,16}.

Of three available mutant *mahogany* alleles, *mg^{3j}* has a stronger effect than *mg* (Fig. 1c) and *mg^L*. To narrow the location of *mg^{3j}*, we constructed a high-resolution genetic map by intercrossing F₁

(CAST/Ei × C3H/HeJ – *mg^{3j}/mg^{3j}*) mice or backcrossing F₁ mice to C3H/HeJ – *mg^{3j}/mg^{3j}* (Fig. 2a). We used DNA probes that closely flanked *mg^{3j}* to construct a physical map using bacterial artificial chromosome (BAC) clones (Fig. 2b, c). Additional genetic mapping narrowed the location of *mg^{3j}* to a single BAC clone, BAC389B9, with an estimated length of 190–200 kilobases (kb) (Fig. 2c).

BAC389B9 was sequenced to >95% completion and analysed for the presence of genes using sequence-similarity searches and gene-prediction programs. Three previously known genes were apparent in the sequence, *Sialoadhesin*, *Centromere autoantigen B* and *Cdc25b* (Fig. 2d). In addition, sequences from eight new potential genes were identified by BLAST analysis: one was related to the gene encoding glial-cell-derived neurotrophic factor receptor-α (provisionally named *Gfra4*); one was related to the ADAM (a disintegrin and metalloproteinase) family of genes; and six corresponded to clusters of anonymous expressed sequence tags (ESTs). Probes from each potential gene were hybridized to RNA samples from tissues of non-mutant, *mg/mg*, and *mg^{3j}/mg^{3j}* mice. As described below, expression of only one of the EST clusters was altered in *mg*, *mg^{3j}* and *mg^L* mice, and we therefore refer to this gene as *Mgca* (for *mahogany candidate*).

The normal *Mgca* transcript in RNA from brain, skin, heart, kidney, liver and lung of non-mutant mice is 9 kb in length, but no expression is detectable in *mg^{3j}/mg^{3j}* mice (Fig. 1a, b). For the 5.5-kb portion of *Mgca* messenger RNA contained within BAC389B9, the sequence was identical between the *mg^{3j}* allele and the chromosome of origin; thus the *mg^{3j}* mutation must affect regulatory sequences that reside in an intron or in 3'-flanking regions. In RNA from *mg^L/mg^L* or *mg/mg* mice, *Mgca* probes detect abnormally large fragments of 10 kb or 9.5 kb, respectively; both alleles also produce low levels of a normal size, 9-kb fragment (Fig. 1a). *Mg* and *mg^L* each contained ~5-kb insertions in adjacent introns of the *Mgca* gene (Fig. 3c); these insertions were not found in non-mutant DNA and therefore explain both the abnormal patterns of RNA expression and the hypomorphic nature of these alleles.

A ~9-kb *Mgca* complementary DNA contig assembled from EST

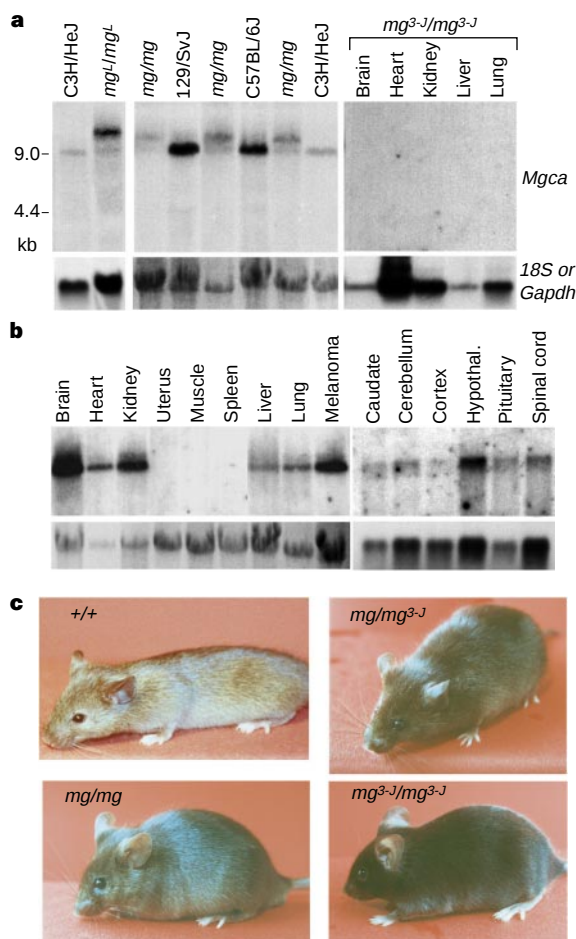


Figure 1 Expression of *Mgca* in wild-type and *mg* mutant mice. **a**, In total brain RNA, northern hybridization analysis with *Mgca* cDNA probes detects a 9-kb fragment from six wild-type strains (C3H/HeJ, 129/SvJ and C57BL/6J shown; DBA/2J, FVB/N and C3H/FeJ not shown), but a 10-kb fragment and reduced levels of the 9-kb fragment from *mg^L/mg^L* mice and a 9.5-kb fragment and reduced levels of the 9-kb fragment from *mg/mg* mice. No expression is detectable in RNA from the indicated tissues of *mg^{3j}/mg^{3j}* mice. **b**, Pattern of *Mgca* expression in RNA from the tissues shown (top panels). Each lane contains 20 µg of total RNA; loading was verified by staining of 18S rRNA with ethidium bromide and/or hybridization to a *Gapdh* control probe (bottom panels). **c**, Coat-colour phenotypes of *mg* and *mg^{3j}* homozygotes and compound heterozygotes on an *Agouti* (*A/A*) background. The *A mg^{3j}/A mg^{3j}* animals show a similar, but not identical, phenotype to that produced by the loss-of-function allele *nonagouti* (*a+/a+* animals have residual pheomelanin pigment behind the pinnae and their ear and tail skin is non-pigmented).

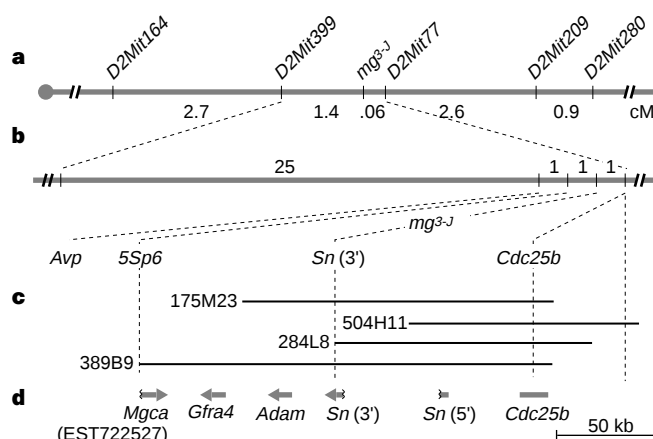


Figure 2 Genetic and physical map of the *mg^{3j}* candidate interval. **a**, **b**, The distance between *mg^{3j}* and closely linked markers is given in centimorgans (cM) (**a**), or by the number of recombinant chromosomes (**b**). A chromosome walk was initiated with probes from the *Avp* and *Cdc25b* genes, which flank *mg^{3j}* and had each previously been shown to lie proximal to D2Mit77²⁹. **c**, Contig of BAC clones isolated with a *Cdc25b* probe. A centromeric end probe isolated from BAC389B9, 5Sp6, mapped proximal to *mg^{3j}*, showing that the *mg^{3j}* mutation must lie within this BAC. The size of the BAC clones was estimated by pulsed-field gel electrophoresis. **d**, The sequence of BAC389B9 contains, at its centromeric end, five exons that encode the 3' 5.5 kb of *Mgca* cDNA, of which EST722527 corresponds to the terminal 0.8 kb. This BAC also contained the genes shown, the *Centromere autoantigen B* gene (not shown) and five EST clusters represented by IMAGE clones 388220, 807697, 427645, 775311 and 614556 (not shown). *Sn*, *Sialoadhesin*.

clones and products of reverse transcription with polymerase chain reaction (RT-PCR) encoded a predicted 1,428-residue type I transmembrane protein (Fig. 3). The extracellular domain of the predicted *Mgca* protein is 93% identical to attractin, a human serum glycoprotein secreted from activated T cells that has been implicated in monocyte spreading and T-cell clustering, and is encoded by a 4-

kb cDNA^{13,14}. The peptide sequence of attractin terminates six codons after it diverges from *Mgca* and eleven codons before the *Mgca* predicted transmembrane domain. (Fig. 3). A probe corresponding to the cDNA encoding the amino-terminal region of mouse *Mgca* (probe a, Fig. 3b) detects two main mRNA isoforms, of 8.5 kb and 4 kb, in human tissues. The 8.5-kb isoform is likely to

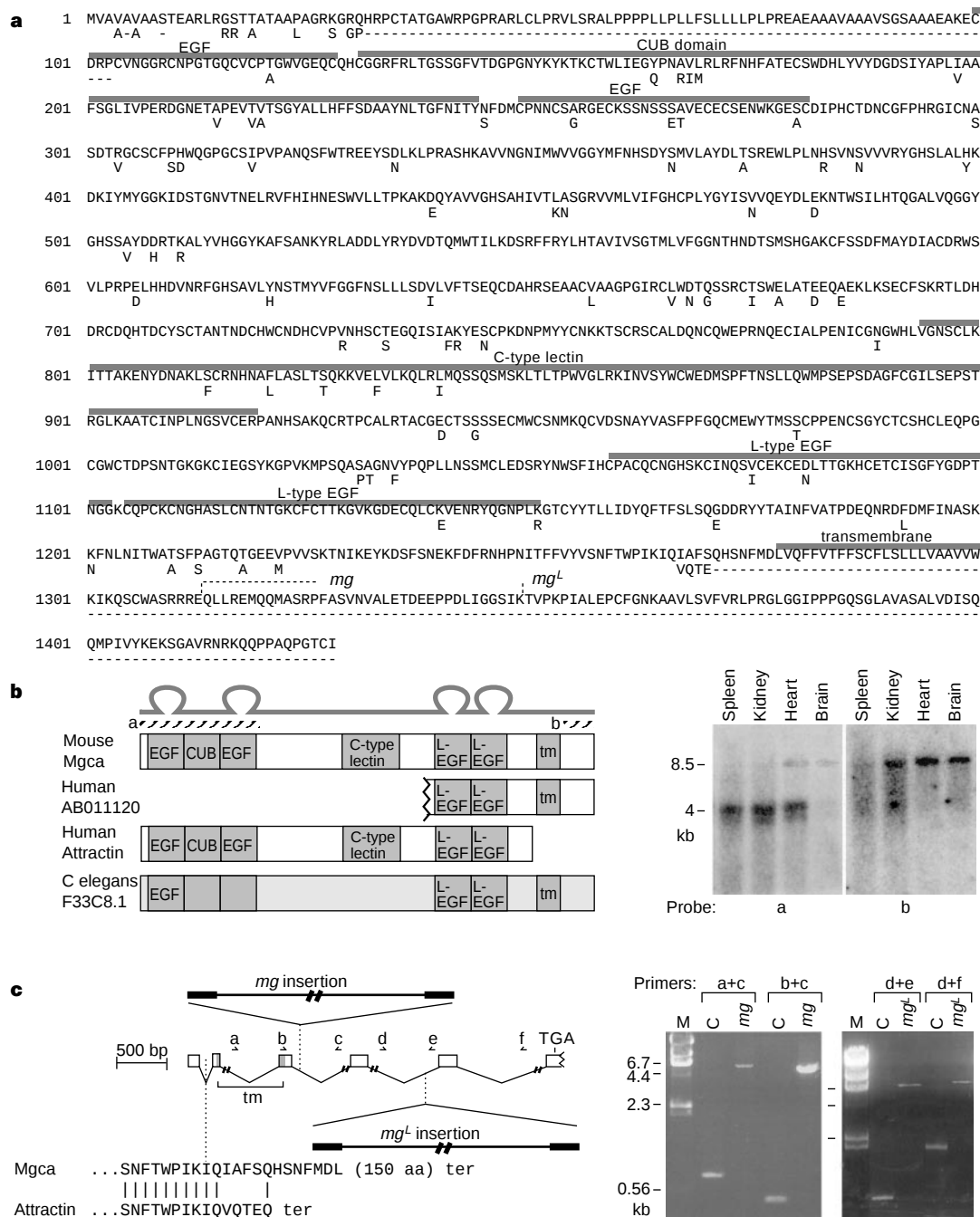


Figure 3 Predicted protein sequence of *Mgca* and location of *mahogany* mutations. **a**, The full-length *Mgca* amino-acid sequence is shown on the upper lines, the lower lines show substitutions or deletions (dashes) in attractin. Compared with *Mgca*, the original attractin sequence¹³ lacks a 72-residue region near the N terminus; more recently, isoforms of attractin have been isolated (GenBank accession number AF106861) in which the 72-residue region is present. **b**, Left, domain structure of mouse *Mgca* and human attractin compared with the domain structure of a protein encoded by the open reading frame of a partial human brain cDNA (GenBank accession number AB011120) and the hypothetical *C. elegans* protein F33C8.1. Right, northern hybridization to RNA

from the indicated human tissues, using a probe common to the *Mgca* and *attractin* cDNAs (probe a), detects 8.5-kb and 4-kb fragments. A probe corresponding to the C-terminal region of mouse *Mgca* (probe b) detects the 8.5-kb but not the 4-kb fragment. **c**, Left, protein-coding sequence and genomic structure of the six 3' exons of *Mgca* and the locations of primers (a-f) used to amplify ~5-kb insertions in the *mg* and *mg^L* alleles. The locations and sizes of the insertions were verified by genomic Southern hybridization (not shown). TM, region encoding the transmembrane domain. Right, PCR-amplification products recovered with the indicated primers, using genomic DNA from C3H/HeJ (C), *mg/mg* or *mg^L/mg^L* mice.

encode the transmembrane form of human attractin because it is also detected by a probe corresponding to the *Mgca* carboxy terminus (probe b, Fig. 3b). In addition, a partial human brain cDNA clone¹⁷, AB011120, encodes a predicted protein that is 97% identical to the C-terminal 452 residues of *Mgca*, and the site at which the attractin and *Mgca*/AB011120 cDNAs diverge corresponds to a splice junction in the *Mgca* genomic sequence (Fig. 3b, c). Thus, the different C-terminal ends of attractin and *Mgca* probably reflect the use of alternative 3' splice acceptor sites.

Attractin and *Mgca* contain, near their N termini, two epidermal growth factor (EGF) domains flanking a CUB domain, a cytokine-receptor signature motif (data not shown) and a C-type lectin domain in the central region, and two laminin-type EGF domains close to the predicted transmembrane domain of *Mgca* (Fig. 3a, b). Attractin and *Mgca* also share several domains with the hypothetical *Caenorhabditis elegans* perlecan-like protein F33C8.1 (Fig. 3b). The

CUB domain is found in a functionally diverse set of developmentally regulated proteins^{18,19}, and the C-type lectin and EGF domains are characteristic of cell-surface heparan sulphate proteoglycans²⁰. There are several potential glycosaminoglycan attachment sites in *Mgca* and F33C8.1, but circulating human attractin lacks glycosaminoglycan side chains²¹.

To determine whether *Mgca* might be required for the secretion, processing or stability of agouti protein, we studied tissue extracts from *mg/mg* and non-mutant animals by western blotting using antiserum against recombinant agouti protein. Native agouti protein from neonatal skin migrates as a band of relative molecular mass 22,000 (M_r 22K) on non-reducing polyacrylamide gels and probably corresponds to a glycosylated, 109-residue fragment, consisting of residues 23–131, that is derived after cleavage of the signal sequence from full-length agouti protein²². We found a fragment of similar size in hypothalamus extracts from *A^y/a* but not *a/a* mice (Fig. 4a). Neither the mobility nor the intensity of this fragment was altered in *A^y mg/a mg* mice compared with *A^y/a+* mice. Thus, *Mgca* does not appear to alter expression of agouti in the hypothalamus of *A^y/a* mice, and is probably required for cell-surface binding of agouti protein and/or for signalling downstream of the melanocortin receptors.

We have shown previously that the melanocortin-1 receptor (Mc1r) is required for the pigmentary effects of *Agouti* and that agouti protein binds directly to Mc1r. From these^{12,23} and our present results, we propose two possible mechanisms of action for *mahogany* protein. First, *mahogany* may function as a low-affinity receptor for Agouti that increases its local cell-surface concentration (model 1, Fig. 4b). The N-terminal domain of agouti protein is glycosylated and very basic, and could interact with the C-type lectin domain and/or potential glycosaminoglycan side chains of *mahogany* protein. Second, *mahogany* protein could be required to desensitize melanocortin receptors (model 2, Fig. 4b) by enabling their agonist-induced internalization or perhaps post-translational modification²⁴. The predicted intracellular domain of *Mgca* is 128 residues in length and lacks any recognizable signalling motifs. However, *mg* has been shown to affect feeding behaviour and metabolism independently of the *A^y* mutation²⁵ (model 3, Fig. 4b). The involvement of *mg* in several aspects of cell signalling, only some of which are specific to agouti protein, is consistent with the relatively widespread expression of *Mgca* and its orthologous relationship with human attractin. Melanocortin agonists have been recognized as potent anti-inflammatory agents^{26,27}; identification of *Mgca* as the transmembrane form of a human immunomodulatory molecule may provide insight into the biochemical mechanism underlying these anti-inflammatory effects. □

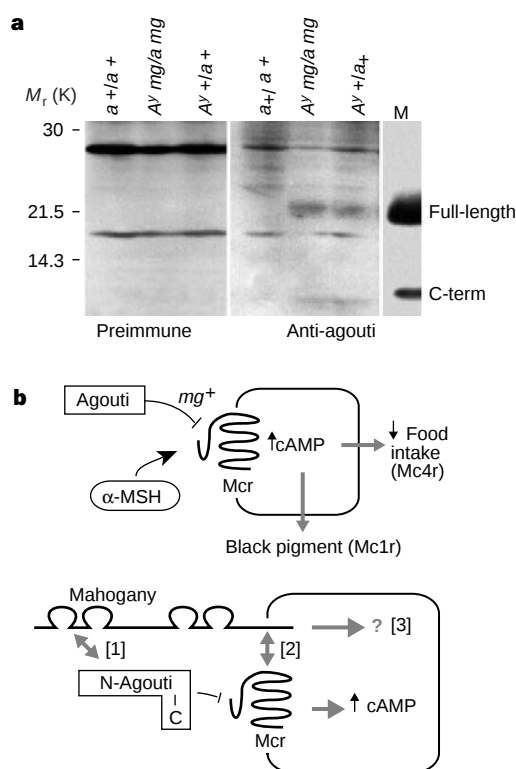


Figure 4 Effect of *mg* on *Agouti* protein and potential mechanisms of action of *mahogany*. **a**, Hypothalamus samples from mice of the indicated genotypes were homogenized in 1% NP40, 0.5 μ g ml⁻¹ leupeptin and 1 mM benzamide at 4°C. Supernatants were mixed with an equal volume of 2x SDS loading buffer, boiled, separated by 15% SDS-PAGE under non-reducing conditions, and then transferred to a nylon membrane. After incubation with primary antiserum at 1:1,000 dilution, immobilized *Agouti* protein was detected with horseradish-peroxidase-conjugated goat-anti rabbit IgG (BioRad) and an enhanced chemiluminescence kit (Amersham). A band of M_r 22K is detected in extracts from *A^y/a+* but not *a/a+* animals, and does not change in mobility or intensity in extracts from *A^y mg/a mg* animals. Preimmune serum taken from the rabbit before antibody production does not detect this band. Full-length (H23–C131) and C-terminal (S73–C131) agouti proteins²² were run on the same gel. M, marker. **b**, Top, we showed previously that *mg* is downstream of *A^y* and upstream of *Mc1r*, and that all of the effects of *agouti* on pigmentation are mediated through *Mc1r*, and that *mg* does not affect circulating levels of α -melanocortin-stimulating hormone^{12,23}. Bottom, thus *mahogany* protein could function as a low-affinity receptor for *Agouti* protein [1]; alternatively, *mahogany* could be required for agonist-induced desensitization of melanocortin receptors [2]. *Mahogany* is also likely to have functions that are independent of *Agouti* signalling²⁵ [3].

Methods

Mice. The *mg* mutation arose on an unknown *agouti* background¹⁶ that, on the basis of the allele sizes of closely linked simple-sequence-length polymorphism (SSLP) markers¹², is likely to be closely related or identical to C3H/HeJ. *Mg* has been backcrossed into this background for 5–10 generations. The *mg^L* (*mahogany* Leicester) mutation arose on the C3H/HeJ background and has a hypomorphic pigmentation phenotype identical to that of *mg*. The *mg^{3J}* mutation is also maintained on the C3H/HeJ background but arose on C3HeB/FeJ.

Genomics. We first typed 818 intercross and 91 backcross animals for chromosome markers D2Mit164 and D2Mit280. Animals that carried recombinant chromosomes were typed for extra internal markers as shown in Fig. 2, and, when necessary, were progeny-tested to distinguish between the *+/+* and *+/mg^{3J}* genotypes. A *Cdc25b* probe²⁸ identified four BAC clones (Research Genetics); BAC ends isolated by vectorette PCR were sequenced and hybridized back to the clones and to key recombinant animals to yield the map shown in Fig. 2. Random insert M13 clones of BAC389B9 were sequenced to roughly sevenfold redundancy and assembled into thirteen contigs covering 205 kb; *Mgca* was identified as an EST cluster represented by IMAGE clones 722527, 653298, 719870, 554708 and 603964. A 9,379-bp *Mgca* cDNA contig

was assembled from IMAGE clone A1043161 (base pairs (bp) 1–1,214 of the *Mgca* cDNA contig) and from multiple, independent products resulting from RT-PCR or rapid amplification of cloned ends (bp 546–9,379) of brain RNA; the protein-coding sequence shown in Fig. 3 corresponds to bp 27–4,311 of the *Mgca* cDNA (GenBank accession number AF119821). The last five exons of *Mgca* (Fig. 3b), bp 3,825–9,379, are contained in BAC389B9. Probes used for northern hybridization analysis were a ~1-kb *Xho*I fragment from A1043161 (Fig. 1a, left, and probe a in Fig. 3b), a 470-bp fragment derived by RT-PCR (bp 3,913–4,382; probe b in Fig. 3b), or the ~700 3'-terminal residues of the *Mgca* 3' untranslated region (Fig. 1a, middle and right, and Fig. 1b) derived from EST722527. Primers used to amplify the *mg* and *mg^L* insertions shown in Fig. 3 correspond to nucleotides 6,550–6,570 (a), 6,883–6,903 (b) and 7,274–7,294 (c) in AF119822, and to residues 1,409–1,427 (d), 2,248–2,266 (e) and 3,266–3,284 (f) in AF119823.

Detection of agouti protein. Polyclonal antibodies to recombinant mouse agouti protein (consisting of residues H23–C131) were generated in rabbits and recovered at day 70. The antiserum used detects 1 ng per lane of the immunogen against which it was raised, as well as 1 ng per lane of C-terminal agouti protein (residues 573–C131) produced by enzymatic cleavage *in vitro*, and recognizes epitopes in both the N terminus and the cysteine-rich C terminus of agouti²².

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A protective role for protease-activated receptors in the airways

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The protection of cells in the upper intestine against digestion by pancreatic trypsin depends on the prostanoid prostaglandin E_2 (PGE₂) and is mediated by protease-activated receptors in the epithelium^{1,2}. As the airway epithelium is morphologically similar and also expresses one of these receptors, PAR2 (ref. 3), and is a major source of PGE₂ (ref. 4), we reasoned that bronchial epithelial PAR2 might also participate in prostanoid-dependent cytoprotection in the airways. Here we show that activation of PAR2, which co-localizes immunohistochemically with trypsin(ogen) in airway epithelium, causes the relaxation of airway preparations from mouse, rat, guinea-pig and humans by the release of a cyclooxygenase product from the epithelium. This physiological protective response in isolated airways also occurred in anaesthetized rats, where activation of PAR2 caused a marked and prolonged inhibition of bronchoconstriction. After desensitization of PAR2, the response to trypsin recovered rapidly by mechanisms dependent on *de novo* synthesis and trafficking of proteins. Our results indicate that trypsin released from the epithelium can initiate powerful bronchoprotection in the airways by activation of epithelial PAR2.

Protease-activated receptors (PARs) are self-activated by tethered peptide ligands that become exposed after extracellular aminoterminal cleavage by thrombin (PAR1, PAR3 and PAR4) or trypsin (PAR1, PAR2 and PAR4)^{1,5–10}. PAR1, PAR2 and PAR4 can also be activated by synthetic peptides corresponding to their tethered-ligand sequences. PAR1 (ref. 11) and possibly PAR3 and PAR4 are involved in thrombogenesis, mitogenesis and vascular inflammation, but the only known role for PAR2 is in cytoprotection in the gut^{1,2}. Using an antibody directed against the carboxy terminus of mouse PAR2 in conjunction with confocal fluorescence microscopy, we found specific PAR2 immunoreactivity localized to epithelial cells, often focally within the cytoplasm, as well as to smooth-muscle cells and fibroblasts in the submucosa of the mouse bronchus (Fig. 1). Mouse PAR2 tethered-ligand sequence, SLIGRL-NH₂ (ref. 8), and trypsin each caused concentration-dependent, rapid-onset and near-maximal relaxation of mouse bronchial rings contracted with the stable muscarinic agonist carbachol. These relaxations were abolished either by removal of the epithelium or by inhibition of cyclooxygenase (Figs 1, 2). For

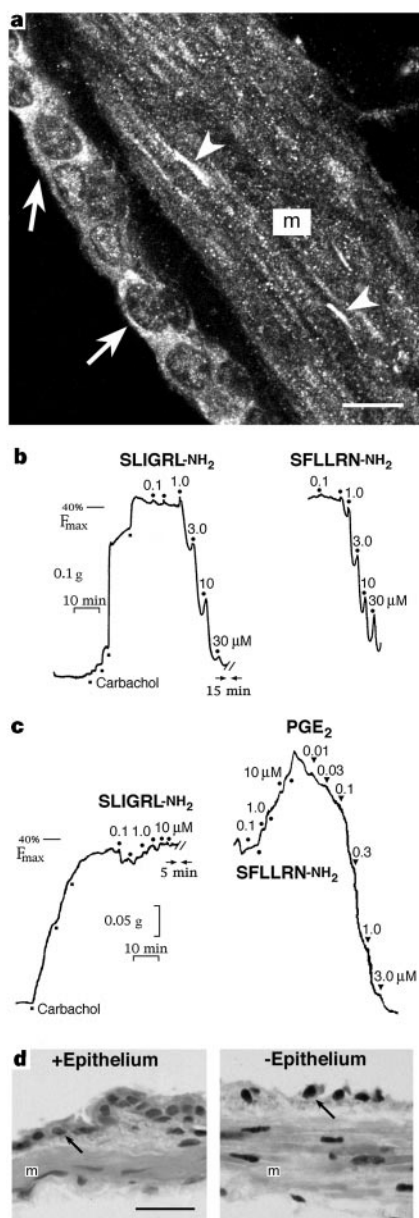


Figure 1 Immunohistochemical localization of PAR2 in mouse bronchi and demonstration that PAR2 and PAR1 mediate epithelium-dependent relaxation in isolated rings of this tissue. **a**, Confocal photomicrograph showing PAR2 immunofluorescence in discrete epithelial cells (arrows) as well as in smooth muscle cells (m) and fibroblasts (arrowheads). In some epithelial cells, the fluorescence appeared to be concentrated within areas of the cytoplasm. Pre-absorption with the peptide sequence used to raise the mouse PAR2 antibody quenched the epithelial, smooth muscle and fibroblast fluorescence (not shown). Scale bar, 10 μ m. **b**, An original, digitized chart recording of changes in isometric force in a single ring of mouse left bronchus with intact epithelium. The tissue was contracted to ~40% F_{max} to acetylcholine (ACh, 30 μ M; see Methods) with cumulative, titrated concentrations of carbachol (note the change of gain and that the force recovered spontaneously over the 15 min break in the trace after maximum relaxation to SLIGRL-NH₂). **c**, Removal of the epithelium with 0.1% Triton X-100 (see Methods) abolished relaxations to SLIGRL-NH₂ and SFLLRN-NH₂, whereas the tissue could still sensitively relax to PGE₂. **d**, Light photomicrographs of cross-sections of mouse bronchi showing that the 0.1% Triton X-100 perfusion technique removes most of the columnar epithelial cells (arrows) with no microscopic evidence of damage to the underlying smooth muscle (m). Scale bar, 30 μ m.

SLIGRL-NH₂, the sensitivity (pEC_{50} , the negative logarithm of the molar concentration that gives 50% of the maximum response) was 5.6 ± 0.1 and the maximum relaxation (R_{max}) was $94 \pm 3\%$. Similar concentration-dependent relaxations were obtained with the PAR1 tethered-ligand sequence SFLLRN-NH₂ (ref. 1) (pEC_{50} , 5.6 ± 0.1 ; R_{max} , $76 \pm 11\%$) and thrombin. In contrast to PAR2 activation, removal of the epithelium or inhibition of cyclooxygenase unmasked smooth-muscle contractions to PAR1 activation with SFLLRN-NH₂ (Figs 1, 2). Unlike SLIGRL-NH₂, which is a specific

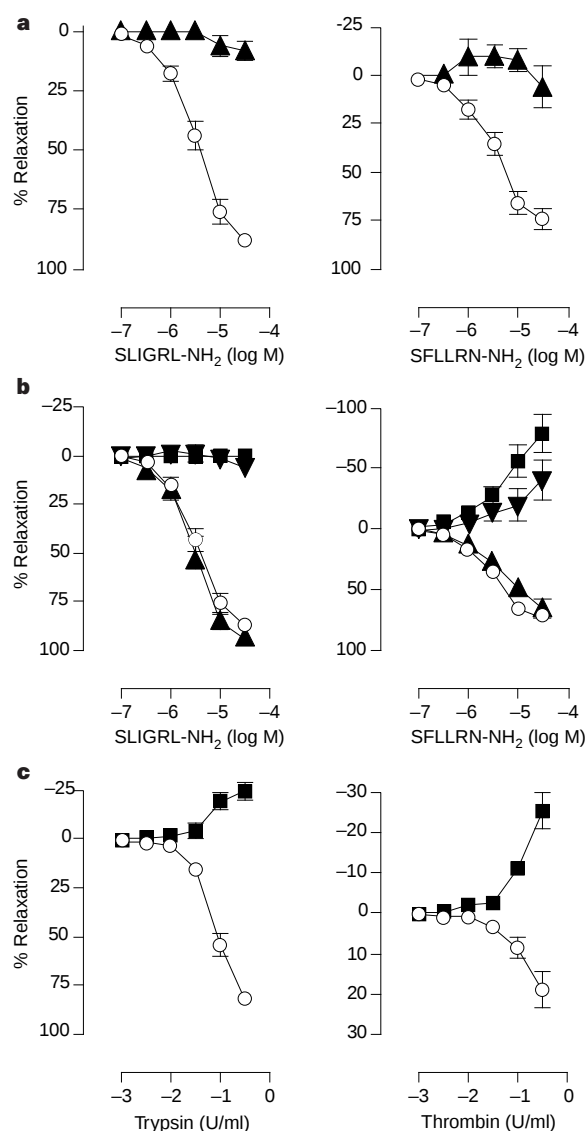


Figure 2 Mechanisms of PAR-mediated bronchial relaxation. **a**, Epithelium- and **b**, cyclooxygenase-dependent relaxations of mouse isolated bronchi to the PAR2 and PAR1 synthetic peptide ligands SLIGRL-NH₂ and SFLLRN-NH₂, respectively. **c**, Relaxations to trypsin and thrombin in epithelium-intact preparations were similarly abolished by cyclooxygenase inhibition. Group data are from experiments similar to that described in Fig. 1, except that tissues were either treated or not treated with indomethacin (3 μ M) and aspirin (100 μ M) to block cyclooxygenase activity, or with a combination of the NO inhibitors L-NOARG (100 μ M) and oxyhaemoglobin (20 μ M). All relaxations and contractions are expressed as percentages of the initial force to carbachol (40% F_{max}), regardless of treatment. Values on the graphs are mean $\pm$ s.e. mean from 5–9 experiments, except aspirin ($n = 3$). Note that the NO inhibitors have no effect on the relaxations to PAR1- and PAR2-activating peptides. O represents control responses; $\blacktriangle$ represents removal of the epithelium in **a**, and inhibition of NO in **b**. $\blacktriangledown$ and $\blacksquare$ represent treatment with aspirin and indomethacin, respectively.

activator of PAR2, SFLLRN-NH₂ can activate both PAR1 and PAR2 (ref. 12). The inability of SLIGRL-NH₂ to contract epithelium-denuded or cyclooxygenase-blocked preparations of the mouse bronchi indicates that SFLLRN-NH₂ causes smooth-muscle contraction by activation of PAR1. Although we cannot rule out a contribution of PAR2 to the epithelium-dependent relaxation induced by SFLLRN-NH₂, the relaxations induced by SLIGRL-NH₂ or low concentrations of trypsin must have been due to activation of epithelial PAR2 or of an unidentified receptor with similar sensitivity to SLIGRL-NH₂ and trypsin. This was confirmed by our observation that responses to SLIGRL-NH₂ were abolished by prior desensitization to trypsin but not to thrombin, whereas those to SFLLRN-NH₂ were abolished following desensitization to both thrombin and trypsin (data not shown). This is consistent with previous reports showing that thrombin activates only PAR1 but that higher concentrations of trypsin can activate both PAR2 and PAR1 (refs 5, 13).

Relaxations induced by SLIGRL-NH₂ and SFLLRN-NH₂ in mouse bronchi were not due to nitric oxide (NO; ref. 14), because they were unaffected by the NO-synthase inhibitor, N^G-nitro-L-arginine (100 μM), and by the NO scavenger oxyhaemoglobin (20 μM; Fig. 2)¹⁵. A prostanoid, rather than NO, must therefore mediate the relaxations in response to both PARs. We found that PGE₂, the most prevalent prostanoid released from the airway epithelium⁴, could powerfully relax mouse bronchi (pEC₅₀, 8.2 ± 0.1; R_{max}, 100%; Fig. 1).

Smaller intrapulmonary airways are likely to contribute more than larger airways to resistance to flow in the lungs. We therefore investigated the effects of PAR-activating peptides in first-generation branches of the mouse main bronchi and found similar indomethacin-sensitive relaxations in response to the PAR ligands in these preparations, although the sensitivity and maximum relaxation to both SFLLRN-NH₂ (pEC₅₀, 5.5 ± 0.02; R_{max}, 58 ± 10%) and SLIGRL-NH₂ (pEC₅₀, 5.1 ± 0.05; R_{max}, 58 ± 4%) were significantly less (*P* < 0.05) than those in the main bronchi (Fig. 2).

As enzymic activation of PARs is irreversible, rapid resensitization is critical for maintaining tissue responsiveness to PAR-activating proteases. Turnover of cloned PAR2 expressed in selected cell lines is rapid and depends on *de novo* synthesis of new protein as well as on trafficking of pre-formed receptors from intracellular

pools^{1,16}. Our results show that PAR2-mediated relaxations in mouse bronchi returned 45 min after desensitization to trypsin (Fig. 3). This recovery was prevented by the protein-trafficking inhibitor brefeldin A (10 μM) or the translation inhibitor cycloheximide (70 μM; Fig. 3). These findings, and our demonstration that PAR2 immunoreactivity is often localized in discrete cytoplasmic regions of airway epithelial cells (Fig. 1), indicate that there is rapid PAR2 turnover from intracellular stores in airway epithelium. We could not demonstrate localization of PAR2 messenger RNA to mouse bronchi by *in situ* hybridization, although PAR2 mRNA was detected in this issue by reverse transcription-polymerase chain reaction (J.R.H., T.M.C., A.G.E., J. Morrison and M. O'Bryan, unpublished results). This apparent discrepancy may be explained by the continual replenishing of intracellular stores of PAR2 (Fig. 1) with stable mRNA of low abundance. The capacity of airway epithelial cells *in situ* to rapidly recover their sensitivity to PAR2 agonists following receptor desensitization indicates that epithelial PAR2 participates in bronchoprotection.

We also observed PAR2-mediated bronchorelaxation in the airways of species other than mouse. SLIGRL-NH₂ caused epithelium-dependent and indomethacin-sensitive relaxations in rat isolated main (pEC₅₀, 5.5 ± 0.1; R_{max}, 56 ± 5%) and second-order (pEC₅₀, 5.1 ± 0.1; R_{max}, 67 ± 5%) bronchi; it also caused similar-potency (pEC₅₀, 5.4 ± 0.2) epithelium-dependent relaxation in guinea-pig

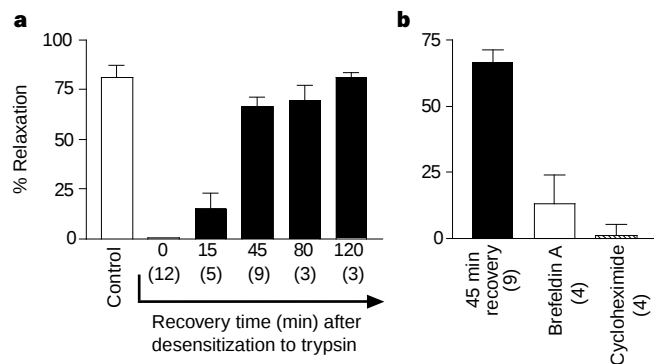


Figure 3 Recovery of responsiveness to PAR2 activation after trypsin desensitization in the mouse bronchus. **a**, Responsiveness to trypsin (0.3 U ml⁻¹) recovered to ~70% of control levels 45 min after the desensitizing concentration of trypsin (0.3 U ml⁻¹) was washed from the bath (see Methods). Time control responses to trypsin at 15, 45, 80 and 120 min recovery were not significantly different from the initial control (not shown). **b**, Recovery of trypsin sensitivity at 45 min was abolished by the protein-trafficking inhibitor brefeldin A (10 μM) or by the translation inhibitor cycloheximide (70 μM). Neither compound had any effect on time control responses to trypsin. Values are mean ± s.e.m. from 3–12 experiments.

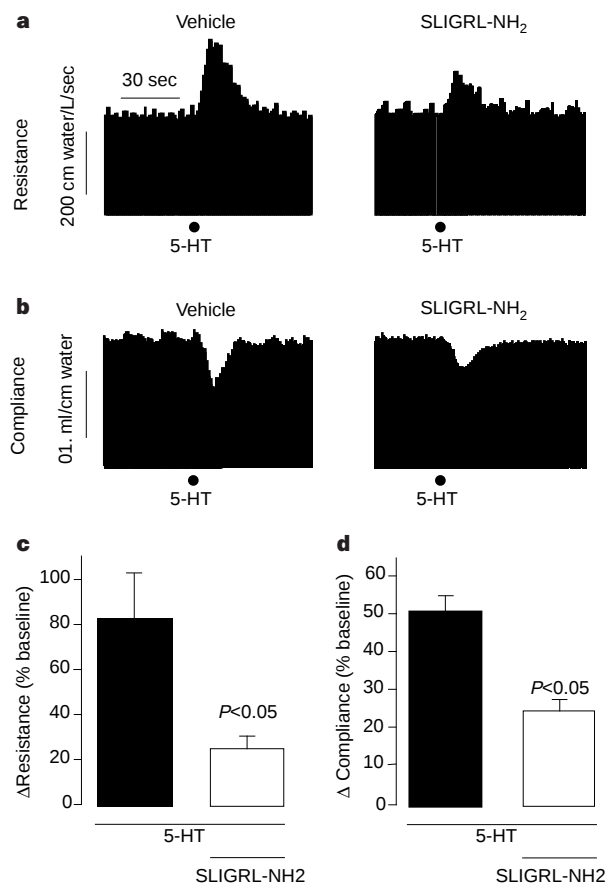


Figure 4 Demonstration that the PAR2-activating peptide SLIGRL-NH₂ inhibits bronchoconstriction *in vivo*. Original chart recordings (**a**, **b**) and grouped data (**c**, **d**) show the effect of a 30-s exposure to an aerosol of a 0.1 mg ml⁻¹ solution of SLIGRL-NH₂ on 5-HT (3 nmol kg⁻¹, i.v.)-induced changes in airway resistance (R_L; **a**, **c**) and dynamic compliance (C_{dyn}; **b**, **d**) in the anaesthetized rat. Complete inhibition of bronchoconstriction to 5-HT lasting for at least 45 min occurred when SLIGRL-NH₂ was used at 1 mg ml⁻¹ (results not shown). Values are mean ± s.e. from *n* = 3 experiments.

isolated bronchi, but with a significantly ($P < 0.05$) lower $R_{\max}$ ($31 \pm 5\%$) than in rat and mouse bronchi. We find PAR2-mediated relaxations in human intrapulmonary airways that are weaker than in mice but are also blocked by indomethacin (T.M.C., J.D.M. and J.M.C., unpublished data; $n = 4$).

We have shown that SLIGRL-NH₂ is an effective inhibitor of bronchoconstriction *in vivo*: 30 s exposure to an aerosol of SLIGRL-NH₂ (0.1 mg ml^{-1}), but not to the scrambled peptide sequence LSIGRL-NH₂, inhibits (50–70%) 5-hydroxytryptamine (5-HT)-induced changes in airway resistance (R_L) and dynamic compliance (C_{dyn}) in anaesthetized rats (Fig. 4). This effect of SLIGRL-NH₂ is overcome by higher doses of 5-HT (data not shown).

The PAR-mediated bronchorelaxation described here is cyclooxygenase-dependent, with PGE₂ being a likely candidate. Substance P also induces epithelium-dependent bronchorelaxation in rat bronchi through release of PGE₂ from the epithelium¹⁷. PGE₂ exerts other bronchoprotective effects in humans at concentrations well below those required for bronchodilation⁴, including inhibition of cholinergic neurotransmission, lung mast-cell activation, eosinophil chemotaxis, interleukin-2 production by T lymphocytes and interleukin-4-induced IgE production by B lymphocytes⁴. PGE₂ synthesized by human airway epithelium may contribute to a refractory response to histamine in humans¹⁸ and to exercise-induced asthma¹⁹. Therefore, although inhalation of PGE₂ can cause acute cough²⁰, stimulation of endogenous PGE₂ release by PAR2 may be crucial for airway defence.

Our results indicate that PAR2 activation causes bronchodilation *in vivo* and epithelium-dependent bronchial relaxation *in vitro*, without direct contraction. As airway epithelial PAR2 is bronchoprotective, it may not have an obligatory proinflammatory role. PAR2 is expressed in the subepithelium, particularly on smooth-muscle cells³, so a dual-compartment model may explain the role of PAR2 in the airways, in which the epithelium acts as a barrier to

separate epithelial cells from the underlying tissues: epithelial and subepithelial PAR2 could be differentially regulated by specific tryptic enzymes released in each compartment (epithelial trypsin and subepithelial mast-cell tryptase). Our proposal that trypsin is the endogenous activator of epithelial PAR2 is supported by the colocalization of trypsin(ogen)²¹ with PAR2 in human airway epithelium (Fig. 5). In addition, trypsin is regulated by α_1 -antitrypsin in the lungs²², whereas there are no known inhibitors of mast-cell tryptase. Our model predicts that epithelial PAR2 normally overrides any proinflammatory effects of subepithelial PAR2 activation and that disruption of the epithelial barrier compromises the normal balance between the two compartments.

We conclude that activation of PAR2 initiates important paracrine protection in the airways by antagonizing increased airway tone. If PGE₂ is the mediator of this effect, then airway epithelial PAR2 have the potential to initiate other paracrine protective responses as well as autocrine protective effects within the epithelium. As such, these intriguing receptors offer scope for designing new treatments for diseases like asthma and bronchitis. □

Methods

In vitro studies. Main bronchi and their first-order branches of specific pathogen-free (SPF) BALB/c mice (15–20 g; either sex), Hartley tricolour guinea-pigs (300–400 g; male) and Sprague-Dawley rats (200–350 g; either sex), all killed by either cervical dislocation or overdosed (i.p.) with sodium pentobarbitone, were placed in cold Krebs solution (95% O₂, 5% CO₂)¹⁵. Human airway preparations (0.5–1 mm external diameter) were from lungs of cancer patients undergoing thoracotomy. Removal of the epithelium was verified histologically in haematoxylin and eosin-stained 8- μm sections. Ring segments (~2 mm long) of bronchi were mounted in Krebs (37°C) in Mulvany–Halpern myographs (JP, Aarhus, Denmark) to record changes in isometric force¹⁵. Tissues were contracted to 40% of their maximum active force ($F_{\max}$; 30 μM acetylcholine), with carbachol (10–500 nM). Nifedipine (0.3 μM) was added to all mouse and rat tissues to control phasic contractile activity. When the active force to carbachol was stable, tissues were exposed to increasing concentrations of the PAR1- and PAR2-activating enzymes thrombin (bovine serum; Sigma) and trypsin (bovine pancreas; Worthington), respectively, and their synthetic tethered-ligand peptide sequences SFLLRN-NH₂ and SLIGRL-NH₂ (>95% purity; from Auspep, and R. A. Hughes), respectively.

For receptor desensitization, mouse bronchi were allowed to recover to their initial active force to carbachol following relaxations to trypsin (0.001–0.3 U ml⁻¹) or thrombin (0.001–0.3 U ml⁻¹), but with the enzymes still in the myograph chamber. When the force was again steady, they were tested for desensitization with maximum concentrations of trypsin and thrombin (0.3 U ml⁻¹). Unresponsive tissues were then exposed to cumulative concentrations of SLIGRL-NH₂ or SFLLRN-NH₂ (0.1–30 μM).

The time course and mechanism of PAR2 resensitization were determined in mouse bronchi that were either left untreated (time control) after acetylcholine washout, or treated with trypsin (0.3 U ml⁻¹ at 2 min intervals) over 20–30 min. Tissues were then contracted with carbachol to ~40% $F_{\max}$ and exposed to trypsin (0.3 U ml⁻¹) at 0, 15, 45, 80 or 120 min after washout. To investigate PAR2 resensitization after desensitization with trypsin, trypsin-desensitized tissues were either left untreated (control) or treated with brefeldin A (10 μM) or cycloheximide (70 μM) before re-exposure to trypsin (0.3 U ml⁻¹) at 45 min.

In vivo studies. Male Sprague-Dawley rats (8 weeks old) were anaesthetized (xylazine 10 mg kg⁻¹, ketamine 100 mg kg⁻¹ and 50 mg kg⁻¹ each for 30 min thereafter; i.p.) and cannulae placed in the trachea, carotid artery and jugular vein. Spontaneous breathing was stopped by intravenous injection of pancuronium bromide (0.4 mg kg⁻¹ and 0.2 mg kg⁻¹ each for 30 min thereafter) and rats were ventilated (tidal volume, 8 mg kg⁻¹ at 90 breaths per min on a SAR-830 ventilator from CWE). Breath-to-breath measurement of airway resistance (R_L) and dynamic compliance (C_{dyn}) were calculated from flow (Fleisch pneumotachograph) and transpulmonary pressure recordings (PMS800, Mumed). 5-Hydroxytryptamine (5-HT) (0.3 mg kg⁻¹, i.v.) was administered as a bolus at 5-min intervals until changes in R_L and C_{dyn} were reproducible. Before each 5-HT challenge, lungs were hyperinflated once (by

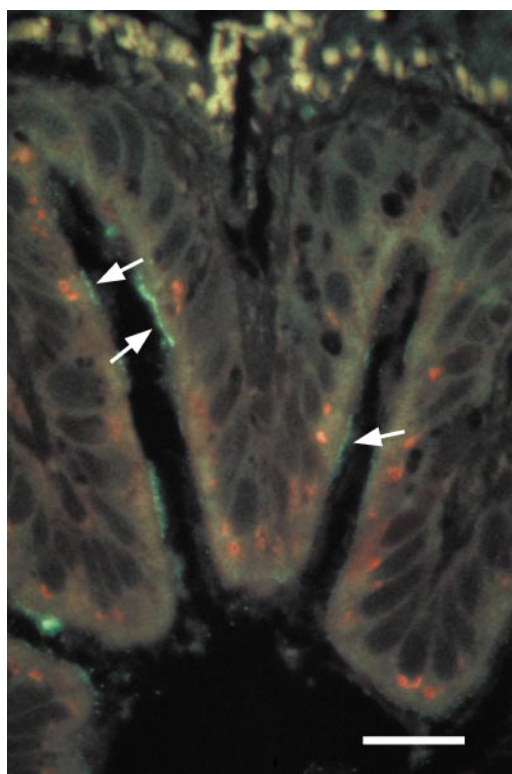


Figure 5 Dual immunohistochemical localization of PAR2 (green) and trypsin(ogen) (red) within the epithelium of a 3- μm -thick cross-section of human intrapulmonary bronchiole. Note that in some cells PAR2 and trypsin(ogen) are coexpressed (arrows). Scale bar, 20 μm .

delivering twice the tidal volume) to prevent and reverse atelectasis. SLIGRL-NH₂, the scrambled peptide LSIGRL-NH₂ (both at 0.1 mg ml⁻¹), and their vehicle controls (saline) were then delivered for 30 s as aerosols generated by an ultrasonic nebulizer (AeroSonic 5000, DeVilbiss) in series with a second ventilator, and the response to 5-HT was determined 5 min later.

Data analysis. Responses were normalized as percentages of the initial active force to carbachol and are presented as mean $\pm$ s.e.m. pEC₅₀ (ref. 15) and maximum response ($R_{\max}$) values were compared by two-tailed unpaired Student's *t*-test or one-way ANOVA with Tukey–Kramer's *t*-tests for multiple comparisons. *P* < 0.05 was accepted as significant.

Mouse immunohistochemistry. Fresh frozen, paraformaldehyde-fixed sections (14 μ m) of mouse bronchi were incubated with rabbit antiserum against the C terminus of mouse PAR2 (CSVKTSY), labelled with a biotinylated donkey anti-rabbit antiserum (Amersham) followed by FITC-conjugated streptavidin (Amersham) and were viewed under a Biorad MRC1000 confocal scanning laser system. There was no staining when the antiserum was preabsorbed with the immunizing peptide sequence.

Human immunochemistry. Paraffin sections (3 μ m) were dewaxed and exposed to the rabbit anti-PAR2 antiserum for 24 h, then a monoclonal mouse antibody against human trypsin(ogen) (Chemicon, MAB1482) was applied. After 24 h, binding of rabbit anti-PAR antiserum was localized as above and trypsin(ogen) was localized using a donkey anti-mouse antiserum conjugated to rhodamine. Expression of PAR2 and trypsin(ogen) was examined under epifluorescence using a Zeiss Axioskop microscope. Scanned photographs of FITC (green) or rhodamine (red) fluorescence were overlaid with Adobe Photoshop software, using reference points to align images.

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Degradation of the cyclin-dependent-kinase inhibitor p27^{Kip1} is instigated by Jab1

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The proliferation of mammalian cells is under strict control, and the cyclin-dependent-kinase inhibitory protein p27^{Kip1} is an essential participant in this regulation both *in vitro* and *in vivo*¹. Although mutations in p27^{Kip1} are rarely found in human tumours, reduced expression of the protein correlates well with poor survival among patients with breast or colorectal carcinomas², suggesting that disruption of the p27^{Kip1} regulatory mechanisms contributes to neoplasia. The abundance of p27^{Kip1} in the cell is determined either at or after translation³, for example as a result of phosphorylation by cyclinE/Cdk2 complexes^{4,5}, degradation by the ubiquitin/proteasome pathway⁶, sequestration by unknown Myc-inducible proteins⁷, binding to cyclinD/Cdk4 complexes⁸, or inactivation by the viral E1A oncoprotein⁹. We have found that a mouse 38K protein (p38) encoded by the *Jab1* gene¹⁰ interacts specifically with p27^{Kip1} and show here that overexpression of p38 in mammalian cells causes the translocation of p27^{Kip1} from the nucleus to the cytoplasm, decreasing the amount of p27^{Kip1} in the cell by accelerating its degradation. Ectopic expression of p38 in mouse fibroblasts partially overcomes p27^{Kip1}-mediated arrest in the G1 phase of the cell cycle and markedly reduces their dependence on serum. Our findings indicate that p38 functions as a negative regulator of p27^{Kip1} by promoting its degradation.

We used a yeast two-hybrid screen¹¹ to identify complementary DNAs from a mouse T-cell lymphoma library encoding proteins that are able to interact with p27^{Kip1}. To obtain genes other than cyclins and cyclin-dependent kinases (CDKs) that were abundant in the library, we used carboxy-terminal amino-acid residues 80–197 of p27^{Kip1} as a bait; these do not contain the CDK-binding domain that is highly conserved among the Cip/Kip family of CDK inhibitors¹². From 3 $\times$ 10⁷ transfectants, three types of cDNA insert, as classified by sequence, were isolated, all of which encoded polypeptides that could interact with full-length p27^{Kip1} as well as with the C-terminal domain of p27^{Kip1} in yeast. The entire coding sequence of one such clone was 91.5% similar to the human *Jab1* cDNA sequence¹⁰ and contained an open reading frame of 335 amino acids encoding a protein of relative molecular mass (M_r) 38K that had three amino-acid substitutions (at positions 129, 333 and 334) compared with the human *Jab1* sequence. We concluded that this clone was the mouse homologue of human *Jab1*. *Jab1* was originally identified as a co-activator of c-Jun and JunD (ref. 10), and contains an MPN domain¹³, which is highly conserved among the Mov34 protein family¹⁴. We have used mouse *Jab1* in our investigation; the other clones will be described elsewhere.

We tested the specificity of the interaction between p38^{Jab1} and p27^{Kip1} *in vitro*. Glutathione S-transferase (GST)-tagged p38^{Jab1} fusion proteins could bind to recombinant p27^{Kip1} proteins, but not to cyclins B1 and E, Cdk2, or a closely related CDK inhibitor, p21^{Cip1} (Fig. 1a). Among GST-fusion proteins containing different regions of p27^{Kip1} (Fig. 1b), p27(1–151) and p27(97–197), but not p27(1–102), were able to bind to *in vitro* translated ³⁵S-labelled p38^{Jab1} (Fig. 1c, top), suggesting that the sequence comprising residues 97–151 of p27^{Kip1} might contain the p38^{Jab1}-interaction surface. We found that p27(Δ 97–151) lacking this region did not bind to p38^{Jab1}. The QT domain (amino acids 144–194 of p27^{Kip1})¹⁵,

which is conserved between p27^{Kip1} and p57^{Kip2}, minimally overlaps with this region (only eight amino acids), and therefore is not involved in interaction with p38^{Jab1}. In control experiments, recombinant cyclinD1/Cdk4 complexes bound to any of the p27-deletion mutants containing the CDK-binding domain, but not with p27(97–197), which lacks this domain (Fig. 1c, bottom). So far, we have been unable to detect a complex between full-length p27^{Kip1} and p38^{Jab1} *in vivo*, even in overexpressing cells. However, a portion of the p27(1–186) deletion mutant lacking the CDK phosphorylation site (Thr 187) was found in a complex with p38^{Jab1} in Cos7 cells ectopically expressing both proteins (Fig. 1d), indicating that phosphorylation of Thr 187 by cyclin/CDK complexes, which triggers downregulation of p27^{Kip1} (refs 4, 5), reduces the stability of p27^{Kip1}–p38^{Jab1} complexes *in vivo*. In agreement with this idea, a complex between p38^{Jab1} and p27(97–197) that lacked the CDK-binding domain was also detected in Cos7 cells (data not shown).

Introduction of a haemagglutinin(HA)-tagged p38^{Jab1} expression vector into proliferating NIH3T3 fibroblasts (transfection efficiency was >70%, as determined by using green fluorescent protein (GFP) as a marker¹⁶ and by anti-HA immunofluorescent staining) markedly reduced the expression of endogenous p27^{Kip1} without significantly affecting that of p21^{Cip1} (Fig. 2a). Furthermore, exogenously expressed HA–p27^{Kip1}, but not HA–p21^{Cip1}, was also downregulated by p38^{Jab1} (Fig. 2b) in a dose-dependent manner (Fig. 2c). The synthesis rates of HA–p27^{Kip1} were almost the same in the presence and absence of p38^{Jab1} (Fig. 2d), but the half-life of HA–p27^{Kip1} was reduced in cells co-expressing p38^{Jab1} (more than 5 hours versus 1.4 hour) (Fig. 2e: compare white and black squares; Table 1). Downregulation of p27^{Kip1} was inhibited in cells pretreated with inhibitors of the 26S proteasome, for example LLnL, MG132 or lactacystin, but not in those treated with a control inhibitor, LLM¹⁷ (Fig. 2f). The stability of p27^{Kip1} significantly decreased in p38^{Jab1}-transfected cells, in which AP-1 activity barely increased, whereas p27^{Kip1} was relatively stable in c-Jun-overexpressing cells with

Table 1 Effect of p38^{Jab1} on half-life of p27^{Kip1} variants

	–p38 ^{Jab1}	+p38 ^{Jab1}
p27(1–197)	>5	1.4 ± 0.4*
p27(1–186)	>5	4.5 ± 0.9*
p27(1–151)	>5	>5
p27(97–197)	>5	>5
p27(Del 97–151)	>5	>5
p27(1–197)NES	>5	ND
p27(T187A)	>5	4.0 ± 1.0*

Half-lives are given in hours in the presence and absence of p38^{Jab1}.

Experiments were repeated 3 times. ND, not determined.

* Data are mean values from 3 independent experiments ± s.d.

higher AP-1 activity (Fig. 2g, h), indicating that, although Jab1 was originally identified as a co-activator of c-Jun and JunD¹⁰, stimulation of AP-1 activity is neither necessary nor sufficient for p27^{Kip1} downregulation. Ectopically expressed p38^{Jab1} therefore downregulates p27^{Kip1} *in vivo* not by affecting its rate of synthesis but by destabilizing it in a 26S proteasome-dependent manner. In our experiments we did not detect the slow-migrating forms of p27^{Kip1}, or p27^{Kip1}–ubiquitin ladders, but this does not preclude the possibility that p27^{Kip1} is ubiquitinated before degradation.

The p27(1–186) deletion mutant was more resistant than full-length p27(1–197) to p38^{Jab1}-mediated downregulation (Fig. 2e: compare filled circles with filled squares; Table 1), which is consistent with our finding that p27(1–186), but not p27(1–197), can complex with p38^{Jab1} (Fig. 1d). Additional deletion of nuclear localization signal sequence rendered the molecule (p27(1–151)) resistant to the action of p38^{Jab1} (Table 1). In addition, the degradation rate of a p27^{Kip1} mutant harbouring alanine instead of threonine at position 187 (p27(T187A)), or of variants lacking either the CDK-binding domain (p27(97–197)) or the p38^{Jab1}-interaction domain (p27(Δ97–151)), was not accelerated by co-expression of p38^{Jab1} (Table 1). These results indicate that, in addition to the binding of p38^{Jab1}, prior transportation into the

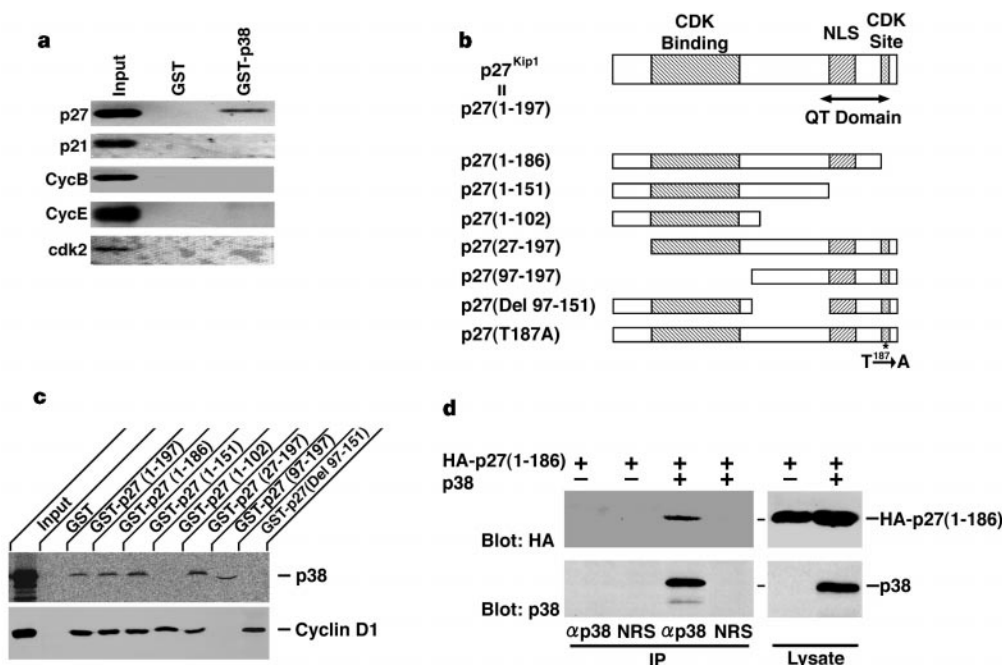


Figure 1 Interaction of p38^{Jab1} with p27^{Kip1}. **a**, p38^{Jab1} specifically interacts with p27^{Kip1} *in vitro*. GST or GST–p38^{Jab1}, immobilized on glutathione beads, was incubated with recombinant CDK inhibitors, cyclins and CDKs. Bound proteins were detected with the appropriate antibodies. **b**, p27^{Kip1} mutants. **c**, *In vitro* interaction of p27^{Kip1} deletion mutants with p38^{Jab1} or cyclinD1/Cdk4 complex. GST or GST–p27 derivatives were incubated with *in vitro* translated [³⁵S]p38^{Jab1} proteins or with recombinant cyclinD1/Cdk4 complex. Bound [³⁵S]p38^{Jab1} was

detected by autoradiography and bound cyclinD1/Cdk4 complex was detected by immunoblotting with antibody against cyclin D1. **d**, *In vivo* association between p38^{Jab1} and the p27 mutant p27(1–186). Cos7 cells were transfected with expression vectors for p38^{Jab1} and/or HA–p27(1–186) (ratio of 7 to 3 for co-transfection) as indicated (top) and collected after 48 h. Specific interaction was analysed by immunoblotting using the antibodies indicated on the left after immunoprecipitation with the antibodies indicated at the bottom.

nucleus and phosphorylation of Thr 187 by cyclin/CDK kinases are required for p27^{Kip1} to be marked out for degradation.

Ectopic HA-p27^{Kip1} expressed in NIH3T3 cells was primarily located in the nucleus (Fig. 3; top row, left). Co-expression of p38^{lab1} markedly decreased the p27^{Kip1}-specific immunofluorescent signals, whereas p21^{Cip1} signals in the nucleus remained the same (data not shown), consistent with the results shown in Fig. 2. A ten-times increased exposure of the cell sample revealed ~80% of the p27^{Kip1} signals in the cytoplasm (Fig. 3, top row, right). Treatment with proteasome inhibitors (LLnL, MG132 or lactacystin) inhibited the p38^{lab1}-mediated translocation and subsequent downregulation of p27^{Kip1} (Fig. 3; second row, right). p27(1–186) and p27(97–197), both of which can bind to p38^{lab1} but are resistant to downregulation (Table 1), were translocated from the nucleus to the cytoplasm by p38^{lab1} (Fig. 3; compare the second and fourth rows on the left with fourth and seventh rows on the right) in a proteasome-inhibitor-sensitive manner (Fig. 3, fifth row, right). p27(Δ97–151), which was unable to bind to p38^{lab1}, remained in the nucleus of cells expressing p38^{lab1} (Fig. 3, eighth row, right). About 80% of p38^{lab1} was located in the nucleus of proliferating cells (Fig. 3, bottom row, left) but the cytoplasmic signals from p38^{lab1} increased as p27 molecules relocated from the nucleus to the cytoplasm (Fig. 3, right). p27(1–151) was stably located in the cytoplasm, regardless of ectopic p38^{lab1} expression (Fig. 3, third row on the left and sixth row on the right). In such cells, p38^{lab1} stayed mainly in the nucleus. Leptomycin B, a

specific inhibitor of NES/CRM1-dependent nuclear export^{18,19}, suppressed transport of p27 by Jab1 to the cytoplasm (Fig. 3, third row, right) and its subsequent downregulation (Fig. 2f). Analysis of the p27(T187A) mutant confirmed that phosphorylation of Thr 187 was essential for p27^{Kip1} degradation but not for cytoplasmic translocation (Fig. 3, bottom row, right), but we do not yet know the function of other potential CDK phosphorylation sites. Cytoplasmic transportation *per se* was not sufficient for p27^{Kip1} to be degraded because full-length p27^{Kip1} fused with a nuclear export signal (NES) sequence derived from MAP kinase kinase²⁰ was efficiently exported into the cytoplasm (Fig. 3, sixth row, left) but its degradation was not significantly accelerated (Table 1). Thus, p38^{lab1} appears to interact with p27^{Kip1} in the nucleus and translocate it to the cytoplasm in a 26S proteasome-dependent manner. p38^{lab1} may accelerate p27^{Kip1} degradation by bringing it to the degradation machinery in the cytoplasm or by facilitating phosphorylation of Thr 187.

To determine the physiological relevance of p38^{lab1}-mediated p27^{Kip1} downregulation *in vivo*, we investigated the effect of p38^{lab1} on G1 arrest induced by p27^{Kip1}. Ectopic introduction of p27^{Kip1} efficiently inhibited entry into S phase of more than 80% of proliferating mouse fibroblasts (Fig. 4a). Co-expression of p38^{lab1} partially but significantly rescued cells from G1 arrest induced by p27^{Kip1} but not by p21^{Cip1} (Fig. 4a). Ectopic overexpression of p38^{lab1} had little effect on BrdU incorporation by proliferating

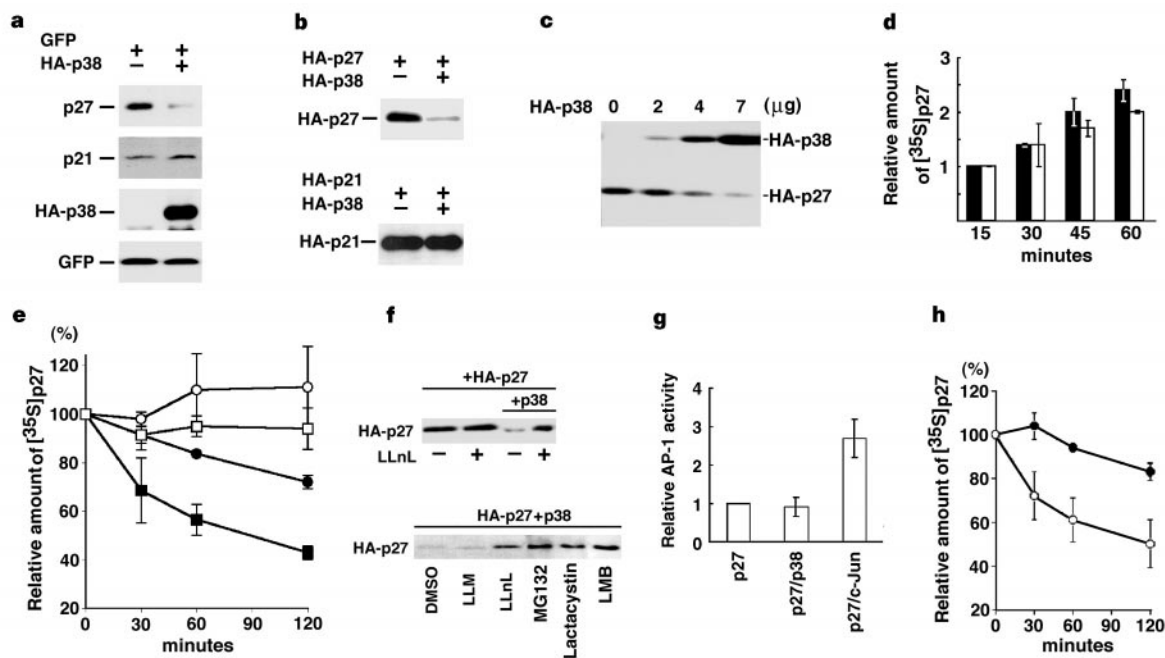


Figure 2 Downregulation of p27^{Kip1} by p38^{lab1}. **a**, Specific downregulation of endogenous p27^{Kip1} by ectopic p38^{lab1}. NIH3T3 cells were transfected with a GFP expression vector together with a p38^{lab1} expression vector (+) or a control plasmid (–). After 48 h, total cell extracts were analysed by immunoblotting using antibodies against p27^{Kip1}, p21^{Cip1}, an HA-epitope, or GFP. Transfection efficiency was ~70%, as determined by GFP expression viewed by fluorescence microscopy and by immunofluorescent staining using antibody against an HA epitope. **b**, p38^{lab1}-mediated specific downregulation of ectopically expressed HA-p27^{Kip1}. NIH3T3 cells were transfected with the vectors indicated at the top (ratio of 1 to 9 for p27^{Kip1} and p38^{lab1} co-transfection) and after 48 h, total cell extracts were analysed by immunoblotting using anti-HA antibody. **c**, Dose-dependent downregulation of p27^{Kip1} by p38^{lab1}. NIH3T3 cells were transfected with a constant amount of HA-p27 (1 μg) and increasing amounts of HA-p38^{lab1} (top). After 48 h, total cell extracts were analysed by immunoblotting using anti-HA antibody. The total amount of plasmid DNA used for each transfection was adjusted to be the same by adding the empty vector. **d**, p38^{lab1}-coexpression has little effect on the rate of p27^{Kip1} synthesis. NIH3T3 cells transfected with HA-

p27^{Kip1}, either alone (black bars) or with HA-p38^{lab1} (white bars), were incubated with [³⁵S]methionine for 15, 30, 45 and 60 min, then collected and the relative ³⁵S activity contained in HA-p27 was counted. **e**, Stability of wild-type and mutant p27^{Kip1} in the presence or absence of p38^{lab1}. NIH3T3 cells transfected with HA-p27 plasmid alone (white symbols) or with HA-p38^{lab1} (black symbols) were pulse-labelled with [³⁵S]methionine for 30 min and chased with excess cold methionine. At the indicated times, cells were collected and the relative ³⁵S in HA-p27 was counted. The results of p27(1–197) (full-length p27^{Kip1}) (squares) and p27(1–186) (circles) are shown. **f**, Downregulation of p27^{Kip1} by p38^{lab1} is sensitive to 26S proteasome inhibitors and LMB. NIH3T3 cells transfected with HA-p27 alone or together with HA-p38^{lab1} were incubated with various reagents (bottom) and expression of HA-p27 was analysed by immunoblotting. **g**, **h**, Increase in AP-1 activity is neither necessary nor sufficient for p38^{lab1}-mediated p27^{Kip1} degradation. NIH3T3 cells were transfected with HA-p27 and AP-1 reporter, together with p38^{lab1} or c-Jun. After 48 h, AP-1 activity contained in the cells was measured (**g**), or cells were pulse-labelled as in **e** and the relative ³⁵S in HA-p27 was counted (**h**). Filled circles, HA-p27 + c-Jun; circles, HA-p27 + p38^{lab1}.

cells, but drove a marked number of serum-starved cells to progress into S phase in a dose-dependent manner (Fig. 4b). This phenotype mirrors that of p27^{Kip1} downregulation by antisense oligonucleotides in BALB/c3T3 cells²¹. Thus, ectopic overexpression of p38^{lab1} both neutralized the inhibitory action of p27^{Kip1} *in vivo* by down-regulating it and reduced the serum dependence of the cells.

The ideas that translocation and subsequent degradation of p27^{Kip1} were due to p38^{lab1}, and not to an indirect effect such as a promotion of cell-cycle progression was confirmed by: (1) cells expressing p27(1–186) together with p38^{lab1} arrest in G1 (data not shown), so cytoplasmic transportation of p27^{Kip1} by p38^{lab1} does not require cell-cycle entry; (2) even at early times after transfection (12

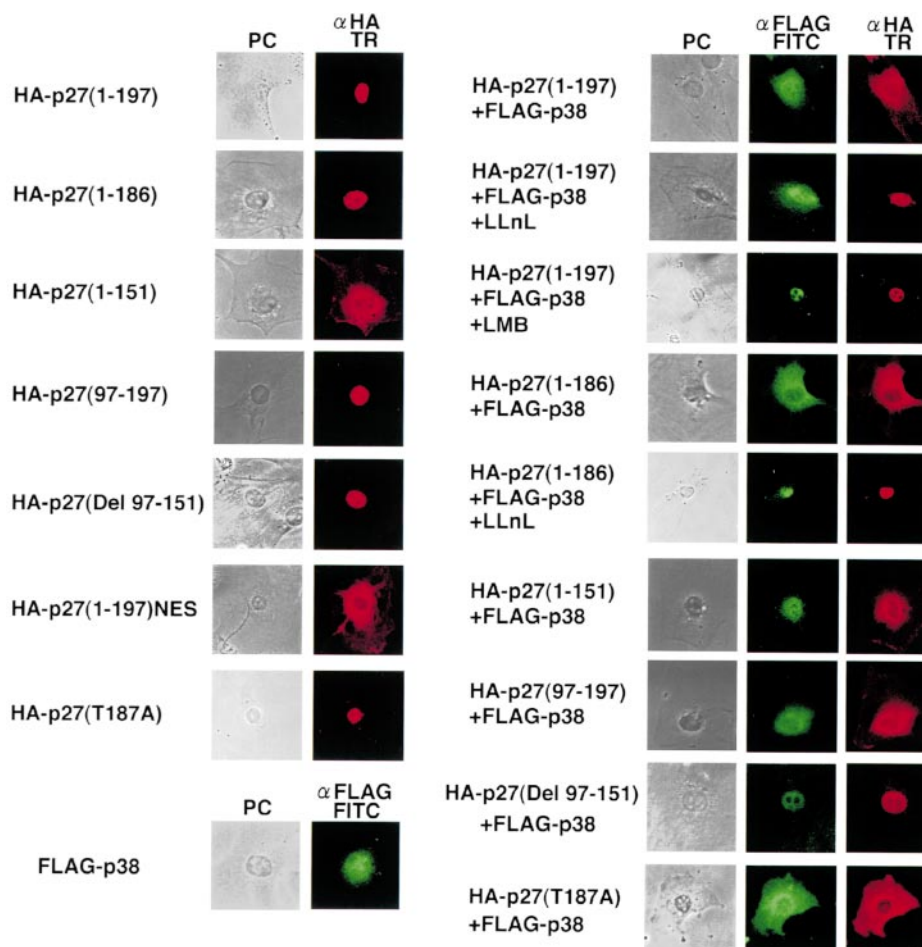


Figure 3 Subcellular localization of p27^{Kip1} variants in the presence and absence of p38^{lab1}. NIH3T3 cells were transfected with the expression vectors shown on the left (ratio of 1 to 9 for p27^{Kip1}/p38^{lab1} co-transfection). After 48 h, they were simultaneously stained with anti-Flag and anti-HA antibodies. In some cases, cells were treated with LLnL or LMB before fixation, as indicated. Quantitative

analysis of cell samples using a confocal microscope showed that the immunofluorescence signals in one cell for HA-p27^{Kip1} were about the same, regardless of subcellular localization, apart from the HA-p27^{Kip1} signal in the presence of p38^{lab1} (top row, right panels; red), which required ~10-times-longer exposure times to obtain the same intensity. PC, phase contrast.

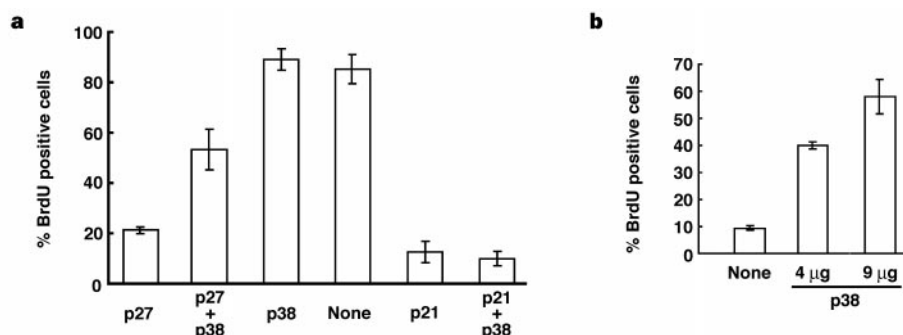


Figure 4 Ectopic p38^{lab1} expression partially overcomes p27^{Kip1}-mediated G1 arrest and reduces the serum dependence of cells. **a**, p38^{lab1} neutralizes inhibition by p27^{Kip1} *in vivo*. NIH3T3 cells were transfected with HA-p27 (or HA-p21 as a control), Flag-p38^{lab1}, or both. After 48 h, cells were incubated with BrdU for 24 h and simultaneously stained with anti-BrdU and anti-HA antibodies. Percentages of BrdU-positive cells among HA-positive cells are shown. In the

case of transfection with p38^{lab1} alone, anti-Flag antibody was used instead of anti-HA antibody. **b**, p38^{lab1} reduces serum dependence of murine fibroblasts. NIH3T3 cells were transfected with HA-p38^{lab1}, serum-starved for 48 h, incubated with BrdU for 24 h and simultaneously stained with anti-BrdU and anti-HA antibodies. Percentages of BrdU-positive cells among HA-positive cells are shown.

hours post-transfection) when most transfectants were still in the cycle, we obtained the same results as those shown in Table 1, indicating that ectopic p27^{Kip1} is stable and that co-expression of p38^{Jab1} decreases its stability (data not shown).

p38^{Jab1} has been identified as a component of the 450K COP9/signalosome complex^{13,22–24} which phosphorylates IκB, the NF-κB precursor and c-Jun (ref. 22), and is structurally similar to the proteasome regulatory complex and the eIF3 translation-initiation factor complex^{13,22–24}, so they may have common substrate-binding sites²² or interact in a similar way with the proteasome core particle¹³. Although the precise role of this complex in p27^{Kip1} regulation remains unclear, our identification of p38^{Jab1} as a specifically binding, negative regulator of p27^{Kip1} should help explain how the cell-cycle-dependent proteolytic machinery is regulated so that it can select key cell-cycle regulators for degradation. □

Methods

Yeast two-hybrid screen. C-terminal residues 80–197 of p27^{Kip1} were fused in-frame to the GAL4 DNA-binding domain using the pAS2 vector²⁵. The resulting 'bait' plasmid (pAS2-p27C) was used to screen a pACT-mouse T-cell lymphoma library (Clontech) by the yeast two-hybrid method in Y190 yeast cells^{11,25}, as described²⁶. Briefly, yeast transfectants were cultured on Trp[−] Leu[−] His[−] selection medium and the resultant colonies were tested for β-galactosidase activity. After segregation, plasmids containing cDNA inserts were recovered from yeast cells and tested to see whether they could confer His-requirement in Y190 cells when transformed with the plasmid pAS2-p27C and not with an empty pAS2 vector. The entire coding sequence of the mouse *Jab1* cDNA (Genbank accession number AF068223) was isolated from a mouse embryonic cDNA library (Novagen) by standard plaque-hybridization methods.

Recombinant proteins and *in vitro* protein-binding assay. cDNA fragments corresponding to the different regions of p27^{Kip1} were amplified by PCR and sequenced to confirm sequence integrity. cDNA fragments were inserted into pGEX (Pharmacia) in-frame with GST. GST fusion proteins were expressed in bacteria and purified as described²⁷. Crude cell extracts containing recombinant mammalian CDK inhibitors, cyclins and CDKs expressed in Sf9 cells by infection with baculovirus expression vectors were prepared as described²⁸. A pBluescript plasmid containing the entire coding sequence of *Jab1* was transcribed and translated *in vitro* using a TNT T7/T3-coupled reticulocyte lysate systems kit (Promega) according to the manufacturer's instructions. Binding was done in buffer containing 50 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM DTT and 0.1% Tween 20, and protein complexes were washed in the same buffer.

Cell culture and high-efficiency transfection. NIH3T3 and Cos7 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The entire coding sequence of p38^{Jab1} and different regions of p27^{Kip1} cDNA were inserted into the expression vector, pCG-N-BL (designed by M. Tanaka²⁹ and modified by J. Fujisawa), which contains SV40 origins of replication and can express HA-tagged exogenous cDNA under the control of the cytomegalovirus (CMV) promoter. p38^{Jab1} cDNA was inserted into the pFlag-CMV-2 expression vector (Eastman Kodak) in-frame with a Flag epitope. Synthetic oligonucleotides corresponding to the NES sequence of MAPKK²⁰ were inserted between an HA tag and the coding sequence of p27^{Kip1} to generate an HA-p27(1–197)NES vector. Cells were transfected with expression vectors (usually at a ratio of 1 to 9 for p27^{Kip1} and p38^{Jab1} co-transfection; total amounts of plasmid DNAs used for each transfection were adjusted to be the same with the empty vector) by a modified calcium phosphate–DNA precipitation method³⁰. We optimized efficiency by varying the pH of the buffer from 6.8 to 7.1 in 0.05 increments and the amount of plasmid from 5 to 15 µg per 35-mm plate in 1-µg increments for each preparation. We always found that 50–80% of the transfected cells expressed exogenous proteins encoded in the plasmid, first determined by expression of the co-transfected GFP vector (ratio of 1 to 10) and confirmed by immunofluorescent staining.

Protein analysis. Cell lysis, immunoprecipitation, gel electrophoresis and immunoblotting have been described^{26–28}. For biosynthesis reactions, cells were metabolically labelled with 200 Ci of [³⁵S]methionine per ml (1,000 Ci mmol^{−1};

Tran³⁵S-label; ICN) in methionine-free medium containing 10% dialysed FBS for the times indicated. Polyclonal anti-p27, anti-p21, anti-cyclin B1, anti-cyclin E and anti-Cdk2 antibodies were from Santa Cruz Biotechnology. HA-tagged proteins were detected using mouse monoclonal antibody against an HA-peptide epitope (anti-HA antibody, Clone 12CA5; Boehringer Mannheim). Polyclonal rabbit antibody against mouse p38^{Jab1} was generated against bacterially produced polypeptides.

Immunofluorescent staining. Cells were fixed in 3% paraformaldehyde, permeabilized in 0.5% Triton X-100, stained with anti-HA rabbit polyclonal (BabCO) and anti-Flag mouse monoclonal (Eastman Kodak) antibodies, and incubated with fluorescein (FITC)-linked antimouse and Texas red (TR)-linked antirabbit IgG (Amersham). For determination of BrdU incorporation, cells stained with anti-HA or anti-Flag rabbit polyclonal antibody followed by TR-linked antirabbit IgG (Amersham) were treated with 1.5 M HCl and stained with anti-BrdU mouse monoclonal antibody (Amersham) and FITC-linked antimouse IgG (Amersham). Cell samples were viewed by phase-contrast or fluorescence microscopy. More than 500 cells were counted for each transfectant.

Transactivation assay. To assay AP-1 activity, cells were transfected with the −73 Col-LUC reporter¹⁰ and the CMV-β-gal plasmid, together with additional plasmids as indicated. Luciferase and β-galactosidase activities were determined using reporter gene assay kits (Boehringer Mannheim) according to the manufacturer's instructions. The ratio of luciferase/β-gal activity in p27 transfectants was set to 1.0 relative to AP-1 activity.

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CBP-independent activation of CREM and CREB by the LIM-only protein ACT

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Transcriptional activation by CREB and CREM requires phosphorylation of a serine residue within the activation domain (Ser 133 in CREB; Ser 117 in CREM) which as a result interacts with the coactivator CBP^{1,2}. The activator CREM is highly expressed in male germ cells and is required for post-meiotic gene expression^{2–4}. Using a two-hybrid screen, we have isolated a testis-derived complementary DNA encoding a protein that we term ACT (for activator of CREM in testis), a LIM-only protein which specifically associates with CREM. ACT is expressed coordinately with CREM in a tissue- and developmentally regulated manner. It strongly

stimulates CREM transcriptional activity in yeast and mammalian cells and contains an intrinsic activation function. As ACT bypasses the classical requirements for activation, namely phosphorylation of Ser 117 and interaction with CBP, it represents a new route for transcriptional activation by CREM and CREB. ACT may define a previously undiscovered class of tissue-specific coactivators whose function could be specific for distinct cellular differentiation programmes.

CREB and CREM are transcription factors directly coupled to signalling pathways. Phosphorylation within the P-box, and consequent CBP interaction, turns them into powerful transcriptional activators^{1,2}. The activation domain also comprises two glutamine-rich regions (Q1 and Q2) and interaction of Q2 with the TATA-binding protein (TBP)-associated hTAF130 seems to be involved in activation⁵. Although CREB is ubiquitous, CREM has a remarkable tissue distribution and has important roles in the nuclear response to physiological stimuli². In particular, the activator CREM is 100-fold more abundant in male germ cells than in any other tissue⁴. It controls the transcription of various post-meiotic genes^{2,3} and its targeted inactivation in mouse completely blocks spermatogenesis at early spermatid stage^{6,7}. Unexpectedly, activated CREM is not phosphorylated at Ser 117 in germ cells (L. Monaco and N. Foulkes, manuscript in preparation). Thus, although the physiological role of CREM in germ cells is well established, the molecular mechanism of its function remains elusive.

We investigated how activation by CREM could occur without phosphorylation. A yeast two-hybrid system was used to screen a murine testis cDNA expression library with the CREM activation domain (CREM-AD; Fig. 1a). The CREM activation domain fused to the GAL4 DNA-binding domain is completely inactive in yeast (Fig. 1b, c) as there are no CBP and hTAF130 yeast homologues.

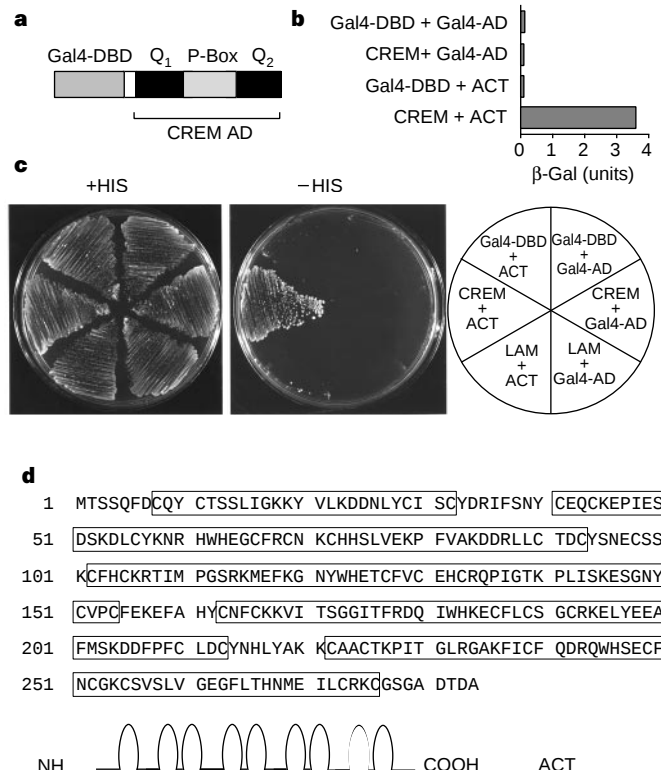


Figure 1 Isolation of a CREM coactivator in testis by yeast two-hybrid assay. **a**, Gal4-DBD/CREM fusion used as bait and the CREM activation domain (AD) structure. Q1 and Q2 are glutamine-rich domains¹². **b**, β-Galactosidase levels in yeast expressing ACT and Gal4-DBD/CREM, bearing the Gal1 upstream activation (UAS)-lacZ reporter. **c**, Growth of transformants coexpressing CREM and ACT on selective medium. CREM corresponds to Gal4-DBD/CREM-AD; ACT

indicates the clone obtained from the screening. Lamin (LAM), Gal4-DBD and Gal4-AD were negative controls. Individual Leu⁺ Trp⁺ transformants were streaked on synthetic medium lacking leucine and tryptophan (+HIS) or leucine, tryptophan, histidine (-HIS). **d**, ACT sequence with boxed LIM domains and schematic representation of ACT structure.

One clone interacted with high affinity when tested for nutritional selection and β -galactosidase activity (Fig. 1b, c). This encodes a previously unknown 284 amino-acid protein (Fig. 1d), ACT (for activator of CREM in testis), containing four complete copies and one amino-terminal half of the LIM motif (Fig. 1d). LIM domains are constituted by double zinc-finger structures, are present in various proteins and are thought to mediate protein-protein interactions^{8,9}.

Expression of the ACT gene is highly specific to the testis (Fig. 2a). We have raised an antibody specific to ACT which revealed a protein of the expected size (relative molecular mass, M_r , 33K). ACT protein colocalizes in purified spermatids with CREM¹⁰ (Fig. 2b). *In situ* analysis shows regulated expression during germ-cell differentiation (Fig. 2c), coordinated with CREM¹⁰. Immunohistochemistry demonstrates that ACT protein is nuclear and is expressed in round and elongated spermatids (Fig. 2d). Thus, CREM and ACT proteins are coproduced *in vivo*.

ACT and CREM proteins associate efficiently (Fig. 3). Purified full-length ACT fused to glutathione-S-transferase (GST) was tested for binding to different CREM isoforms. The presence of the sole P-box is sufficient for binding ACT (Fig. 3a). Consistent with the two-hybrid assay, ACT does not bind to the repressor ICER¹¹, indicating that the DNA-binding domain is dispensable for interaction. To map the region involved in CREM association, we generated ACT truncations in each LIM motif. Mutants lacking the fourth LIM domain (GST-ACT 1–221) or the N-terminal half LIM domain (GST-ACT 38–284) interact with CREM, whereas deletion of the two C-terminal LIM domains (GST-ACT 1–162) impairs association (Fig. 3b). Thus, the third LIM domain seems to be critical for interaction between CREM and ACT. Association between CREM and ACT is also revealed by coimmunoprecipitation after expression in mammalian cells (Fig. 3c). Extracts from cells transfected with CREM and Myc-tagged ACT expression vectors, individually or in combination, were immunoprecipitated using anti-CREM antibodies. Western blot analysis revealed that

ACT associates with CREM in immunoprecipitated complexes (Fig. 3c). No ACT protein was coimmunoprecipitated by anti-CREM antibodies when ACT and CREM were expressed separately. Conversely, CREM-ACT association was detected when the Myc-tagged ACT protein was immunoprecipitated from cotransfected cells, using an anti-Myc antibody (Fig. 3c). Finally, the CREM-ACT association is also revealed by coimmunoprecipitation of native proteins from a testis extract (Fig. 3d).

Sequence analysis of the ACT cDNA from the two-hybrid screening revealed that, surprisingly, the coding sequence was not in-frame with the GAL4 activation domain (GAL4-AD) in the yeast expression vector. Thus ACT could be translated from its own AUG and therefore have intrinsic coactivator properties in yeast. To test this, the GAL4-AD was deleted, generating ACT (delGal4-AD) (Fig. 4a). Strikingly, ACT alone powerfully activates CREM-dependent transcription. Thus ACT bypasses the requirement of CBP for activation, because CBP is lacking in yeast. A fusion of ACT in frame with GAL4-AD (ACT/Gal4-AD) elicits only double the activity of ACT, indicating that ACT, *per se*, has an efficient activation potential. To assess whether ACT has an intrinsic activation domain we fused it to the GAL4 DNA-binding domain (ACT/Gal4-DBD). This fusion protein efficiently induces activation (Fig. 4a). Thus, ACT bears an autonomous activation domain as its recruitment to DNA is sufficient to elicit activation.

The CREM gene generates both activators and repressors^{2,12}. We investigated whether ACT can modify function of the repressors. In a two-hybrid assay ACT-mediated coactivation takes place only in the presence of CREM activator isoforms, as the P-box in combination with at least one glutamine-rich domain is required (CREM τ 1 and CREM τ 2, Fig. 4b).

ACT is a powerful activator in mammalian cells. Using a GAL4-based reporter¹³ CREM-mediated activation was enhanced by ACT in a dose-dependent manner. Strikingly, ACT functions in the absence of coexpressed protein kinase A (PKA) catalytic subunit (Fig. 4c). Analogous results were obtained with full-length CREM

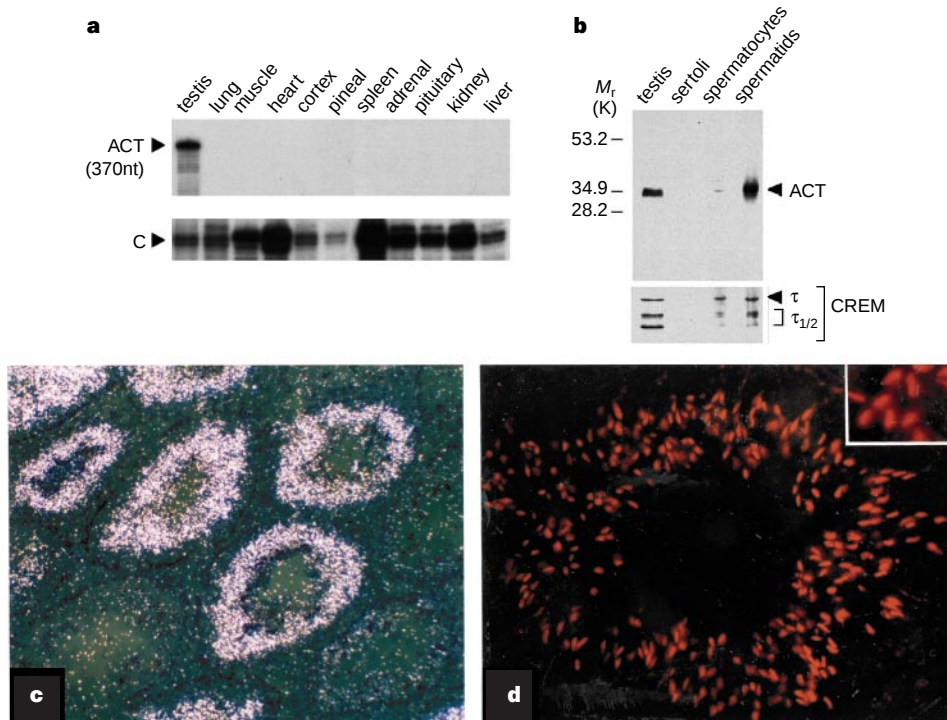


Figure 2 Analysis of ACT expression. **a**, ACT is testis-specific. Total RNA (10 μ g) from mouse tissues analysed by RNase protection assay. C, mouse β -actin internal control. **b**, Western blot using CREM- and ACT-specific antibodies with

purified testis cells. **c**, *In situ* hybridization of ACT mRNA expression in adult mouse testis. **d**, ACT is nuclear in spermatids. Immunohistological analysis of ACT protein in adult mouse testis ($\times 400$) and inset, higher magnification ($\times 1,000$).

and CREB on a CRE-driven somatostatin reporter and the testis-specific angiotensin converting enzyme (ACE) and caldesmon promoters^{14,15} (Fig. 4d, e and data not shown). ACT also displays inherent activating function in mammalian cells, as ACT fused to GAL4-DBD strongly stimulates transcription from the G4CAT reporter (Fig. 4f).

We tested ACT function on CREB because GAL4-DBD fusion proteins containing glutamine-rich activation domains are inactive in yeast¹⁶. ACT noticeably activates CREB-mediated transcription both in yeast and in mammalian cells (Fig. 4g and data not shown).

Phosphorylation at Ser 133 in CREB and Ser 117 in CREM is critical for activation and CBP interaction¹⁷⁻²¹. Because ACT bypasses the CBP requirement (Fig. 4), we wondered whether a Ser > Ala substitution may prevent ACT function. The Ser > Ala 117 mutation did not affect the CREM-ACT association tested by

coimmunoprecipitation after transfection in mammalian cells (Fig. 5a). Strikingly, the Ser > Ala 117 mutation did not decrease the stimulating ACT function, either in yeast or mammalian cells (Fig. 5b, c). Analogous results were obtained with a CREB Ser > Ala 133 mutation (data not shown). Thus, ACT bypasses the phosphorylation requirement for activation.

Remarkable modulations in gene expression underlie the dramatic changes occurring during germ cell differentiation³. Unlike CBP, p300 and hTAF130, which are ubiquitous, ACT shows a stringent tissue-specific and temporal expression coordinated with CREM. Interactions between LIM-only proteins and transcription factors have been described in some differentiating cell systems^{8,22-25}. We provide the first evidence, to our knowledge, that LIM domains can bear an intrinsic activation potential. Moreover, ACT bypasses the classic requirement of activation by CREB

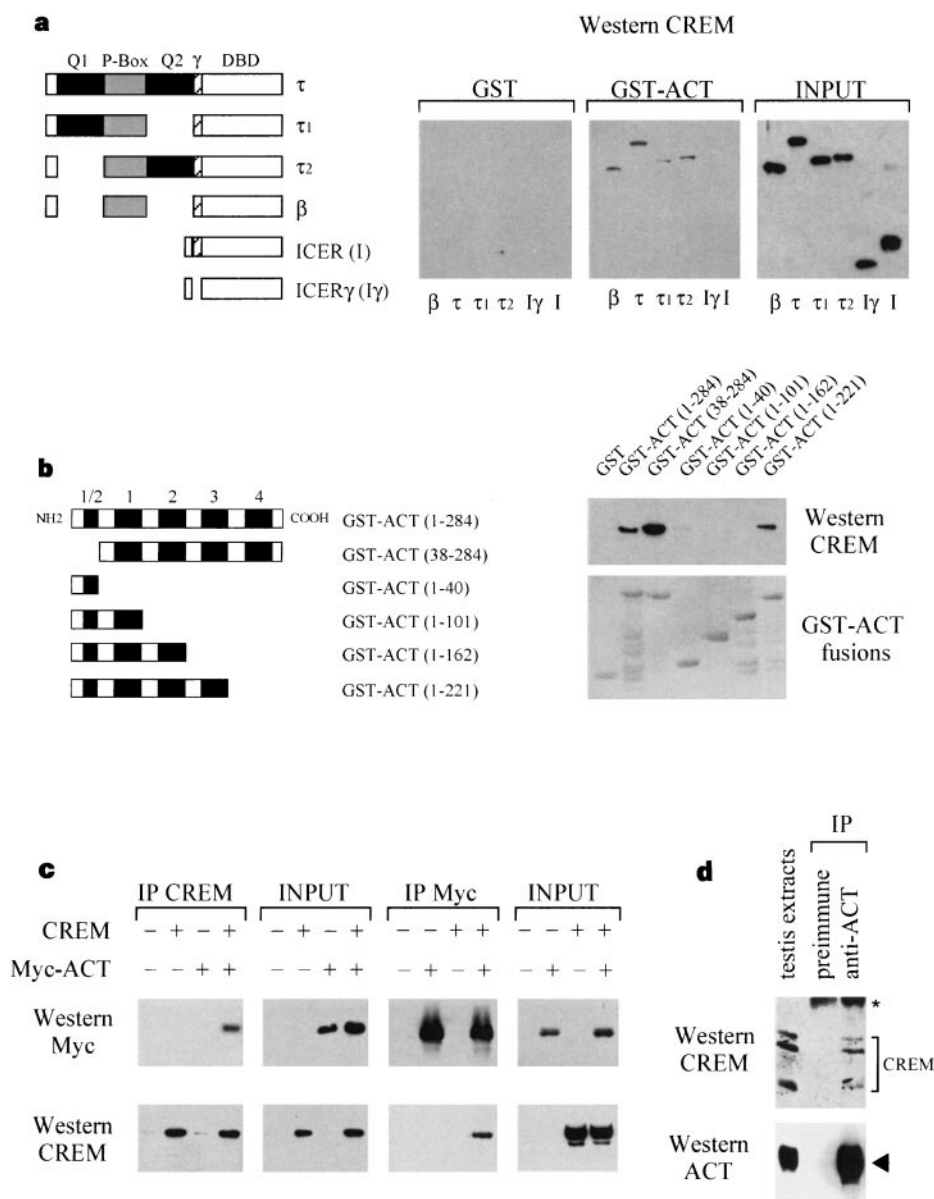
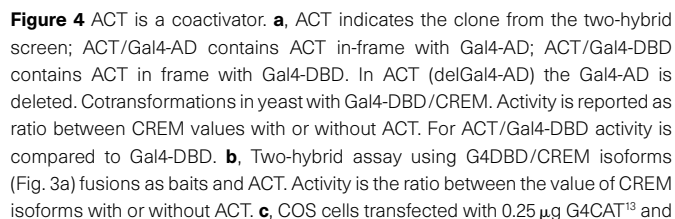


Figure 3 ACT associates with CREM. **a**, ACT binds to the activation domain. Scheme of CREM isoforms¹². CREM proteins incubated with GST-ACT or GST were analysed after GST 'pull-down' by western blot using CREM antibodies. CREM protein aliquots were quantified by western blot. **b**, The third LIM domain is necessary for CREM association. ACT deletions used in GST pull-down; black boxes indicate LIM domains. Equal amounts of truncated proteins (GST-ACT

fusions) or GST were incubated with CREM τ . Blots were probed with CREM antibodies. **c**, ACT coimmunoprecipitates with CREM after transfection in COS cells of CREM and Myc-ACT expression vectors. Immunoprecipitations (IP) with CREM and Myc antibodies revealed by western blot with the reciprocal antibody. **d**, Coimmunoprecipitation of the CREM-ACT complex from testis extracts using the ACT antibody. Bracket, CREM proteins; asterisk, heavy-chain immunoglobulin.



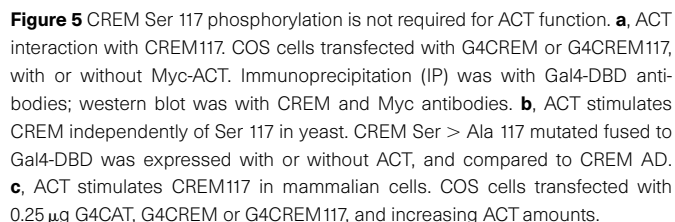
G4CREM with or without pSVPKA¹¹ (0.5 µg), with increasing ACT amounts. CAT values in absence of ACT is 1. **d**, ACT induces the activity of a reporter containing two tandem somatostatin CREs¹¹ in COS cells at levels comparable to pSVPKA coexpression. **e**, Testis-specific ACE and calspermin^{14,15} promoters are activated by ACT in COS cells. **f**, ACT is an activator. G4CAT transfected in COS cells with G4DBD-ACT. Activity without G4DBD-ACT is 1. **g**, ACT potentiates CREB-mediated activation. COS cells transfected with G4CAT (0.25 µg) and G4CREB, with increasing amounts of ACT.

and CREM. Indeed, a striking difference from CBP is that ACT association and function do not require phosphorylation of CREM at Ser 117. The molecular mechanism by which ACT functions is intriguing. It is likely that ACT needs to interact with basal transcription factors or coactivators that must be conserved between yeast and mammalian cells. The absence of a CBP homologue in yeast demonstrates that ACT functions in a CBP-independent manner. Finally, the possible existence of other tissue-specific ACT-like molecules may suggest that ACT represents the first example of a new class of coactivators whose function is specific for distinct steps of cellular differentiation programmes. □

Yeasts. The CREM activation domain (amino acids 1–229) and different fragments of CREM corresponding to functional domains (CREM τ 1 aa1–166; CREM τ 2 1–38/88–229; CREM β 1–38/88–166; CREMQ1 39–87; CREMQ2 168–229) were obtained by PCR and cloned adjacent to Gal4-DBD into pGBT9 (Clontech). The CREB activation domain from pG4CREB Δ LZ²⁶ was cloned in-frame with the Gal4-DBD in pGBT9.

A murine adult testis cDNA library (Clontech) was cotransformed with the pGBT9-CREM bait plasmid in CG1945 yeast cells. Yeast two-hybrid screening was performed as described (Clontech Matchmaker Two-Hybrid System Protocol). β -Galactosidase assay was performed in Y190 yeast cells following the directions of the manufacturer. The results reported are in Miller units and are the means of measurements performed in triplicate from four independent transformations.

Antibodies and western analysis. Rabbit anti-ACT antiserum was prepared by sequential immunization with 100 µg of a peptide spanning amino acids 106–127 of the ACT protein, coupled to keyhole limpet haemocyanin (Pierce). ACT antibodies were purified by an affinity resin containing the same peptide immobilized onto a Sulfolink coupling gel (Pierce). Testis and purified germ cell protein extracts were prepared as previously described¹⁰. Purified ACT



antibodies were used at a dilution of 1:2,000. Immunocomplexes were detected by enhanced chemiluminescence (Pierce).

RNA analysis. Total RNA was prepared and analysed by RNase protection as previously described^{27,28}. The ACT RNA probe (internal fragment from +702 to +1,072 into pBluescript SK⁺) was prepared using an *in vitro* transcription kit (Promega). CREM expression was scored using pN6/1²⁸. In all analyses tRNA was used as a control for nonspecific protection. We used mouse β -actin expression as an internal control for equal RNA loading.

Cell lines and transfections. COS cells (in DMEM, 5% FCS) were plated at a density of 5×10^5 per 6-cm plate and transfected by the calcium phosphate coprecipitation technique. Total amount of transfected DNA was kept constant (10 μ g) by adding pSG5 and pBluescript as necessary. Transfection efficiency was monitored by β -galactosidase assays using the CMV β -gal plasmid. The pG5E4CAT¹³, ACE¹⁴ and calspermin¹⁵ reporters have been described. The expression vectors for ACT, CREM, CREB and the catalytic subunit of PKA are based on pSG5²⁹. G4CREM and pG4CREM117 plasmids have been described¹². Data shown are means of three independent transfections, with inter-experimental variation less than 10%.

Recombinant proteins. To construct GST-ACT fusion proteins, ACT and different deletions (ACT 1–40; ACT 1–101; ACT 1–162; ACT 1–221; ACT 38–284) were obtained by PCR and subcloned in pGex-1XT (Pharmacia). GST fusions were expressed in *Escherichia coli*, extracted in BC0 (20 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 20% glycerol, 1 mM DTT and 0.5 mM PMSF) containing 500 mM KCl and 1% NP-40 and purified on glutathione-Sepharose resin (Pharmacia). GST-protein beads (20 μ l, about 0.5 μ g of protein) were incubated with 100 ng recombinant CREM isoforms at 4°C for 30 min in 500 μ l of BC0 buffer, 200 mM KCl and 0.2% NP-40. Beads were washed and bound proteins were eluted with 20 μ l of SDS loading buffer.

Coimmunoprecipitation assays. Myc-ACT was constructed by inserting ACT in-frame with Myc epitopes into pCS2Myc³⁰. COS cells were transfected with 5 μ g of each plasmid and collected in 1 ml EBC (50 mM Tris-HCl, pH 8.0, 170 mM NaCl, 0.5% NP-40, 50 mM NaF) containing 1 mM PMSF and 10 μ g ml⁻¹ of aprotinin and leupeptin. Following preclearing for 30 min with 50 μ l of a 1:1 slurry of protein A-Sepharose (Pharmacia), supernatants were incubated at 4°C for 3 h with CREM antisera, anti-Myc 9E12 monoclonal antibody or anti GAL4-DBD (Santa Cruz) crosslinked to protein A-Sepharose. Beads were washed in NETN (10 mM Tris-HCl, pH 8.0, 250 mM NaCl, 5 mM EDTA, 0.5% NP-40) and resuspended in 20 μ l of SDS loading buffer for the analysis. Coimmunoprecipitation of the CREM-ACT complex from testis extract was performed using 4-week-old mice. Extracts were prepared in EBC and immunoprecipitation (Fig. 3d) was performed with the anti-ACT antibody.

In situ hybridization and immunocytochemistry. *In situ* hybridization was as described¹⁰. ACT antisense riboprobe was prepared by *in vitro* transcription (Promega). For control of nonspecific signal, consecutive sections were hybridized with ACT sense RNA probe. Immunocytochemical analysis was as described¹⁰. Purified ACT antibodies were used at 1:500 dilution. The secondary antibody (CY3-conjugated anti-rabbit serum; Jackson) was used at 1:1,000 dilution.

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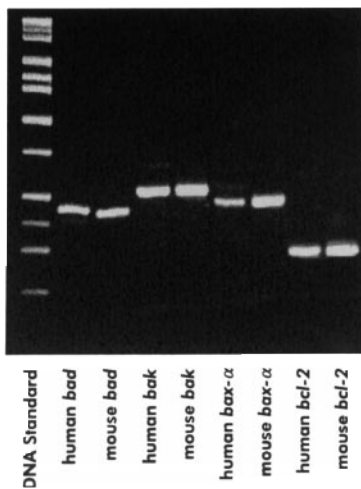
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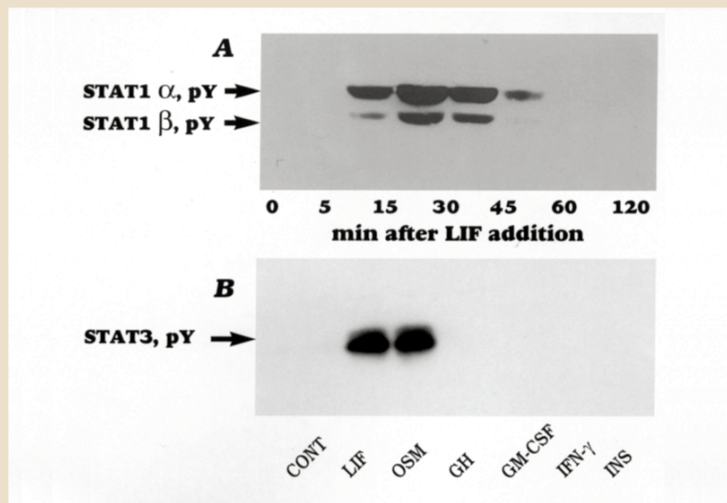
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Phosphatidyl serine translocates from inner to outer cell membrane early in apoptosis

Annexin-V has high affinity for phosphatidyl serine. Genzyme Diagnostics' Annexin V Apoptosis Detection Kit uses the Oregon Green fluorophore and includes the annexin V-Oregon Green conjugate, binding buffer and propidium iodide. The kit helps to differentiate viable cells from cells that are early apoptotic, late apoptotic, or that have disrupted cellular membranes due to necrotic death. The procedure involves brief incubation of viable suspension or adherent cells in a dilution of the annexin V-Oregon Green and propidium iodide followed by flow cytometry, or epifluorescence microscopy.

Reader Service No. 115

Annexin V kit

From Roche

www.roche.com/diagnostics

The Ca^{2+} -dependent phospholipid binding protein, annexin V, again detect ing apoptosis

Roche Diagnostics' new test for identifying cells in early apoptosis exploits the fluorescent red dye Alexa-568. The conjugate of annexin-V and Alexa-568 can be analysed on a 488-nm laser fluorescent analytical cell sorter (FACS) or using a fluorescence microscope. Annexin-Alexa-568 enables rapid characterization of specific apoptotic subpopulations when used as a secondary label with fluorescein-labelled antibodies. In conjunction with a secondary marker, such as Molecular Probes' BOBO-1, it also differentiates necrotic cells from apoptotic cells.

Reader Service No. 116

Annexin V conjugates

From Molecular Probes

www.probes.com

Annexin conjugates for flow cytometry, confocal or epifluorescence microscopy

Working with Nexins Research BV, Molecular Probes produce a range of six annexin V conjugates. The conjugates contain Alexa-488, Alexa-568, Alexa-594, Biotin-X, fluorescein and Oregon Green 488

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to cover a broad range of assay and detection techniques. Two apoptosis assay kits based on annexin are also available (Vybrant kits 1 and 2).

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Signal transduction

Inhibitors

From Sigma www.sigma-sial.com

Inhibitors for signal transduction research

The Sigma range now includes over 1,800 products for signal transduction studies. Included are CNQX, Rab-GDP dissociation inhibitor, apstatin, *O*⁶-benzylguanine, and carmustine. Also available are sotalol, Ras inhibitory peptide, riluzole, and a high-purity preparation of pepstatin A. For convenience of use, Sigma inhibitors are available in a variety of package sizes, including quantities suitable for research or production use.

Reader Service No. 118

U0126

From RBI Natick www.callrbi.com

A specific, non-competitive MEK inhibitor

A new addition to RBI's line of signal transduction agents is U0126. By selectively

inhibiting MEK1 and MEK2 (MAP kinase kinase, MAPKK), U0126 functionally antagonizes AP1-mediated transcriptional activation. By acting as a MEK inhibitor, U0126 can also block the production of a variety of cytokines and metalloproteinases involved in the inflammatory response. The selective action of U0126 should make it a useful tool for the study of protein kinase-mediated signal transduction.

Reader Service No. 119

Electrophoresis

Electrophoresis poster

From Amersham Pharmacia

www.apbiotech.com

Precast gels and electrophoresis platforms explained on a stain-resistant poster

On a double-sided poster, 1 metre wide and 60 cm deep, Amersham Pharmacia Biotech summarize their product range in electrophoresis. The techniques covered include 2-D, SDS-PAGE, native-PAGE and IEF. Also covered are the various DNA techniques, including SSCP, RAPD and DDRT. Electrophoresis platforms covered are the Multiphor II, PhastSystem, GenPhor and IPGpor.

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Pre-cast gels

From Bio-Rad

www.bio-rad.com

Precast gels no in single packs

By making their range of pre-cast electrophoresis gels available in single packs, Bio-Rad aim to reduce waste due to shelf-life expiry. 'Ready Gels' are available as Tris-HCl for PAGE, Tris-Tricine for peptides, TBE for DNA and IEF for pI determination.

Reader Service No. 121

Electrophoresis medium

From Trevigen

www.trevigen.com

A guide to DNA analysis

TreviGel 500 is an electrophoresis medium for the separation and analysis of DNA fragments. Now a complete Applications Guide is available through Trevigen's website, or direct from the company. Topics covered include DNA retrieval for ligation and cloning and high voltage electrophoresis. Contact Trevigen at (800) 873-8443, or on info@trevigen.com.

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These notes are compiled in the Nature office from information provided by the manufacturers. For more details, fill in the reader service card.

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